# Surveillance and Spatial Characterization of *Aedes aegypti* in Sint Eustatius, Netherlands Antilles

by

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# DEDICATION

This thesis is dedicated to my beautiful wife, Sheri, who sometimes was beside me, occasionally behind me providing a push, but always there with love and support.

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

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# ABSTRACT

Surveillance and Spatial Characterization of Aedes aegypti in Sint Eustatius, Netherlands Antilles:

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Dengue, an arbovirus transmitted by *Aedes* mosquitoes, threatens over 40% of the world's population. In Latin America and the Caribbean the incidence and severity of disease has increased rapidly in recent years. On the island of Sint Eustatius, a Caribbean island in the Netherlands Antilles, it has been over 65 years since the last known entomological survey. This study was designed to characterize the domestic distribution of adult and larval *Ae. aegypti* and identify potential risk factors for the distribution of *Ae. aegypti* and dengue infections in Sint Eustatius. Surveys were completed using Prokopack aspiration, BioGents-Sentinel™ mosquito trap, and sampling of larval positive containers. Over 75% of the homes surveyed were positive for *Ae. aegypti*. There were statistically significant positive linear correlations between Prokopack aspiration indoors and BioGents-Sentinel™ mosquito traps outdoors, while no linear correlation existed with either and larval surveys. Garbage related and domestic use containers contributed 95% of the sample larvae sampled, while homes without door and window screens contributed to 82% of the sampled adults. There were no dengue positive mosquito pools identified with qPCR for the study period of June – August 2012.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER 1: Introduction and Background

## INTRODUCTION

In South and Central America and the Caribbean, transmission of arthropod-borne viral diseases is an important component of the tropical disease burden. According to Erickson et al. (7), dengue is the most commonly transmitted arthropod-borne virus in the world. Currently, dengue threatens over 2.5 billion people, which is over 40% of the world's population. Approximately 50–100 million infections occur annually (53). Although 70% of the disease burden occurs in South-East Asia and the Western Pacific, parts of Africa and the Eastern Mediterranean have recently experienced numerous outbreaks. In 2010, several countries in Europe reported indigenous transmission. The incidence and severity of disease has increased rapidly in recent years in both Latin America and the Caribbean. Dengue infections exceeded 1.2 million cases in 2008 and 2.2 million cases in 2010 throughout the Americas, South-East Asia and Western Pacific (53). Urbanization, rapid movements of people and goods, favorable climatic conditions, and lack of trained public health staff contribute to the global increase of dengue.

The overarching goal of this project was to characterize the distribution of *Aedes aegypti* on the island of Sint Eustatius using multiple sampling techniques and to identify potential risk factors for the distribution of adult *Ae. aegypti* and dengue infections. Two specific aims were evaluated in an effort to achieve this goal: 1) Examination of sampling efficiency for two specific adult mosquito collection techniques and 2) Characterization of the spatial distribution of *Ae. aegypti* populations in an attempt to determine risk for dengue infection.

## DENGUE VIRUS

Dengue virus belongs to the *Flavivirus* genus of the family Flaviviridae. This genus includes dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), and tick-borne encephalitis virus (TBEV) among other encephalitic viruses. Most of the viruses in this genus are transmitted by arthropods. However, DENV and YFV differ in that humans are not a dead end host. Viral replication in the human may result in a viremia that is high enough to infect susceptible vectors. Unlike the other flaviviruses, the peridomestic dengue viruses have adapted to humans to such extent that humans serve as the reservoir and the amplifying host and therefore are not zoonoses (48).

DENV is a positive-sense single-stranded RNA (ssRNA+) virus. The RNA genome of dengue only encodes ten proteins (Protein Data Bank, 2008). Three of these encoded proteins are structural proteins forming the shape of the virion and function to deliver RNA to target cells. The remaining seven are nonstructural (NS) proteins that function to produce new viruses once inside a cell (28). The structural proteins of mature virions include the capsid protein C, membrane protein M, and envelope protein E. Structurally the E proteins form an icosahedral shaped capsid that encloses the virion. This capsid is attached to the M proteins anchored to the host-cell-derived lipid bilayer. Together the E and M protein structures surround a spherical nucleocapsid core formed by C proteins (31).

During cell infection, the E protein is responsible for cell attachment after which the virion enters the cell through endocytosis. Inside the cell cytoplasm the virion nucleocapsid is uncoated and the RNA molecule is translated with the host cell's

endoplasmic reticulum. NS proteins then replicate the viral genome, and the structural proteins form the genomes into new virions (31).

There are four antigenically distinct serotypes of dengue virus, DENV 1, DENV 2, DENV 3 and DENV 4, which differ slightly in the composition of these viral proteins. The amino acid sequences of these four serotypes only share 62%-67% homology. This could have resulted in them being classified as distinct viruses rather than being grouped as DENV serotypes (15). However, sequencing of each of these serotypes has identified genotypes for each, though the exact number continues to be revised as further sequences become available.

The phylogenetic relationships of DENV 1- 4 have been determined through the analysis of the viral envelope protein using molecular techniques. By performing a phylogenic analysis of all sylvatic and urban DENV serotypes available, it is thought that all serotypes emerged from a common sylvatic ancestor (15). Analysis of the E protein gene sequences for the various DENV strains distributed globally, place the sylvatic serotype of DENV 1, 2, and 4 in the basal position in relation to the corresponding endemic DENV which is consistent with ancestral status and endemic lineages emerging independently from sylvatic progenitors (45). Today DENV is distributed throughout tropical and sub-tropical regions of the world. Studies indicate that certain genotypes are more virulent than others of the same serotype and are more often associated with the risk of severe disease (31). For one particular genotype, Asian DENV-2, there is evidence of a higher human virulence and greater infectivity for *Ae. aegypti* which could lead to its displacement of American DENV-2 in the Americas (48).

The transmission of DENV occurs in two distinct sylvatic and endemic cycles. Sylvatic dengue has a primitive enzootic cycle that involves lower primates and canopy dwelling *Aedes* mosquitoes in Asia and Africa (10). Transmission of sylvatic dengue to humans does occasionally occur in situations where humans are exposed while working or living in or along the border of forests (44). Endemic dengue transmission occurs among human hosts primarily by the urbanized *Ae. aegypti* and peridomestic *Ae. albopictus*. *Aedes aegypti* is the primary vector implicated for dengue transmission and *Ae. albopictus* is a secondary vector though other *Ae.* spp. have been implicated and likely play a role in geographically restricted areas (12).

## HISTORY OF DENGUE

The history of dengue dates back almost two thousand years. A Chinese encyclopedia from between the third to fifth century A.D. details a disease similar to dengue called water poison, which was linked to flying insects associated with water (108). Costa et al. (6) have constructed a 95% highest probability density interval for when the virus first emerged and placed it in the range from 2294 to 1158 years ago and estimated the most common ancestor at 1672 years ago which coincides with the Chinese records. Through phylogenic analysis, all sylvatic and urban DENV serotypes are thought to have emerged separately from a sylvatic ancestor (15). Primitive enzootic transmission cycles of sylvatic DENV involved lower primates in the rain forests of Asia and Africa (10). Sylvatic DENV transmission to humans is limited due to the vectors' host feeding preference and vector competence (15).

The first recognized epidemics of dengue date back to the late 1700's and occurred almost simultaneously on the continents of Asia, Africa, and North America, creating a global dengue pandemic (43). Other major epidemics continued to occur over long intervals, often in 10 to 40 year cycles, prior to World War II. Dengue was often considered a benign, nonfatal disease of visitors to the tropics (11). Even before World War II, studies were conducted to expand the basic knowledge of dengue and the mode of transmission. While dengue may have become endemic in tropical regions during this time, the limitations of human transport between population centers is believed to have been a limiting factor of to the spread of dengue infections.

Following World War II, epidemic dengue hemorrhagic fever emerged in urban centers throughout Asia. The epidemics in Asia were the start of a global pandemic that marked the expansion of DENV's geographic distribution and dengue hemorrhagic fever leading to hyperendemic dengue (11). From 1947 - 1962 a program to eradicate the yellow fever vector was successfully carried out throughout the Americas. When the eradication program ended, however, the vector quickly recolonized and DENV quickly re-emerged (48). By the 1980's, severe dengue returned to the Americas with outbreaks occurring in a similar fashion as had occurred in Asia (11). It has been postulated that the spread and persistence of dengue during this and the last century was due to increased human population, uncontrolled urbanization, and increased international travel (15). In urban and semi-urban areas, transmission has increased in recent years becoming a major international public health concern (53).

## ENDEMIC DENGUE TRANSMISSION

Simply stated, the transmission of dengue virus occurs after the ingestion of viremic blood of a human host by a susceptible mosquito, infection of the blood-fed mosquito and the transmission of the virus to a second human host. During the early phase of the human infection, the virus is present in the circulating human blood, providing a means for mosquito infection and continuation of the cycle. The host viremia required to infect mosquitoes remains at a transmissible level for approximately five days after which time it decreases until the virus is no longer detectable in the blood (46). Viremia measurements in humans are commonly expressed as 50% mosquito infection doses ($MID_{50}$) and ranges from barely detectable to over $10^{8.5}$ $MID_{50}$ in humans (10). According to Halstead, the viremia required in humans to infect mosquitoes has yet to be measured accurately, however, there is evidence of mosquitoes biting a viremic volunteer and becoming infected by a titer of less than 1 plaque-forming unit per 0.2ml (13).

After a female mosquito takes a blood meal from a viremic host and the dengue virus enters the mosquito, the virus will require an additional 8-12 day extrinsic incubation period before it can be transmitted to another human (57). The dengue virus begins replication in the cells lining the mosquito midgut before disseminating into the hemolymph to infect other tissues (22). Four barriers to infection are present, the midgut infection barrier, the midgut escape barrier, the salivary gland infection barrier and the salivary gland escape barrier. Vector competence varies as the prevalence of these barriers varies in natural populations of *Ae. aegypti* (23). While this author indicates the variance in the prevalence of these barriers, it is more likely the variance in the effectiveness of the barriers as the barriers are present in all mosquitoes.

About 7-10 days post-infection the virus reaches its peak concentration in the midgut and after 12-18 days the peak concentration is reached in the salivary glands (57). Once replication begins in the salivary glands the mosquito remains infective for the remainder of its life, which can range from a few days to a few weeks. The length of the extrinsic incubation period is dependent upon the host viremia, strain of mosquito, genotype and lineage of virus as well as environmental factors such as ambient temperature and humidity (35). In addition to transmission associated with feeding behavior, vertical transmission may exist between females and progeny, though it is unclear whether the mechanism is transovarial or by infection of the mature egg at the time of oviposition. This would offer an additional reservoir for virus maintenance (15).

Dengue is transmitted to humans by the saliva that enters human skin via the mouthparts of an infected mosquito during probing for and taking of a blood meal. Every time an infectious *Ae. aegypti* imbibes a blood meal or probes into a human, transmission of the virus can occur (29). According to Whitehorn (49), while there is a poor understanding of the initial events during human infection, dendritic cells in the dermis appear to be the target for infection. Once the cells are infected they migrate to the lymphatic system, which leads to the infection of the macrophage and monocyte cells, serving to amplify the infection. Dissemination from the lymphatic system into the vascular system allows for the circulation of the virus and further replication in cells of other tissues. Once the virus begins circulation in the vascular system the infected human is now viremic. When the viremia is at a high enough level the sufficient virus can be ingested by another female mosquito to start the cycle over again. Following an intrinsic

incubation period of 3–8 days after an infectious bite, a human may develop an asymptomatic infection, a sub clinical infection or present with clinical disease (24).

## HUMAN DISEASE

The clinical manifestations of dengue can present as an undifferentiated illness, classic dengue fever, dengue hemorrhagic fever, or dengue shock syndrome. Recovery from infection by one serotype provides lifelong immunity against that particular serotype; however, cross-immunity to the other serotypes after recovery is only partial and temporary. The risk of developing severe dengue is increased with subsequent infections by other serotypes. Dengue fever is a biphasic febrile illness lasting 2-7 days. Symptoms are characterized by fever, headache, retro-orbital pain, malaise, myalgia, and the severe arthralgia that earned this disease the name "break-bone fever" (4). Clinical dengue infections progress through three phases: the febrile phase with viremia, the critical phase where symptoms of severe dengue occur, and the convalescence phase marking recovery.

Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) were first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. Today the World Health Organization identifies these manifestations as severe dengue. Severe dengue can be found throughout most Asian and Latin American countries. It is a leading cause of hospitalization and death among children in these regions. In the Americas in 2010, 1.6 million cases of dengue were reported of which 49,000 cases were severe dengue (53). The risk factors for developing severe dengue include age (the very young and very old), ethnicity and genetic background, acquired or inherited immunity, time between infections, sequence of infecting serotypes, and the viral genotype though,

the pathogenic mechanisms are poorly understood (15). Additionally, herd immunity, which is the collective immunity of a population, plays a role not only in the transmission cycle of DENV, but also the introduction of a second genotype of the same serotype into a population (49).

Dengue hemorrhagic fever is characterized with general signs and symptoms consistent with dengue fever such as a fever that lasts from 2 to 7 days. Symptoms including persistent vomiting, severe abdominal pain, and difficulty breathing may develop as the fever declines. These symptoms mark the beginning of a 24- to 48-hour period when the capillaries become excessively permeable, allowing the effusion from the blood vessels into the peritoneum (causing ascites) and pleural cavity (4). A patient with DHF or severe dengue will have a low platelet count and hemorrhagic manifestations, tendency to bruise easily, bleeding from nose or gums, and possibly internal bleeding. Dengue shock syndrome occurs when the effusion from the circulatory system is not corrected, leading to the failure of the circulatory system and shock, followed by death (4).

### DIAGNOSIS AND DETECTION

Given the epidemic nature of dengue and different clinical manifestations, general clinical laboratory tests and laboratory diagnostics provide for clinical management of patients and confirmation of the virus. The very basic level of care of hospitalized dengue cases includes the use of complete blood count (i.e. hematocrit), for diagnosis and clinical management. Changes in the complete blood count and hematocrit in severe dengue cases signal the onset of the critical phase and plasma leakage (56). Gubler (10) states,

"A definitive diagnosis can only be made in the laboratory," that is, laboratory diagnostics are required for dengue confirmation.

In addition to the clinical confirmation of dengue, efficient and accurate laboratory diagnostics are important for surveillance, epidemiologic investigations, research, and vaccine development (56). Laboratory diagnostics for virology consist of direct and indirect detection methods. Direct methods consist of viral isolation, genome detection, and antigen detection. Indirect methods consist of serologic diagnosis for antibodies. While a range of diagnostic methods exists, the method chosen will depend on the purpose, time, cost, and facilities (56). Four laboratory diagnostic techniques are commonly used: enzyme-linked immunosorbent assay (ELISA), plaque reduction neutralization test (PRNT), polymerase chain reaction (PCR), and immunofluorescence assay (IFA).

ELISA is a serologic diagnostic test that can be used as a direct method for detection of antigens or as an indirect method for the detection of antibodies. Generally, ELISAs are performed in 96-well plates with one well as a positive-control and one as a negative-control and the remaining 94 wells for samples. The function of ELISA is to determine if antibodies to the virus are present or to determine the amount of viral protein that is bound to an antibody (antigen). To quantify the amount of antibodies present in each well, a series of steps are performed to produce a colored product that is measured for optical density. The production of the colored product begins with the addition of antibodies in cleared serum to the wells of the plate. The antibodies in the serum then bind to proteins coated on the bottom of wells. Enzymes attached to secondary antibodies are then added to the wells containing the serum and incubated. After

incubation a series of rinses with buffer are performed. The final step is the addition of a conjugated enzyme substrate to produce the colorametric product.

The ability of ELISA to detect dengue viral antigens or dengue antibodies IgM and IgG allows for detection of a current dengue infection or evidence of past exposure. Until recently, ELISA did not allow for the determination of serotype; however, NS1 ELISA can detect serotypes (30). The advantage of ELISA is that it is highly sensitive (> 90%), produces quick results and can be performed by laboratories that already perform ELISA for other viruses (12). For serological diagnosis ELISA offers a simple and inexpensive assay; however, consideration must be given to time of antibody formation and the number of dengue infections (22).

Plaque reduction neutralization test is a serological assay to quantify the reduction of virus infectivity. Serial dilutions of serum with a standardized amount of virus are added to a monolayer of virus susceptible host cells on a plate. The cells are covered with a semi-solid medium and allowed to produce plaques. The plaques are areas of infected cells surrounded by uninfected cells. The infected cells are destroyed creating areas that are clear or opaque in the culture medium that have defined borders with the uninfected host cells. The plaques may be visible to the naked eye or require the use of a microscope or staining in order to visualize. Given the concentrations and the count of plaques formed, the end point titers can be calculated for reduction of virus activity. PRNT is considered the gold standard to quantify neutralizing antibodies and determine DENV serotype (42). While PRNT may be the gold standard assay, there are limits to its use including the time and labor required, thus limiting large-scale use in vaccine trials and surveillance (54).

PCR is a molecular detection technique that can take as little as a single strand of RNA or DNA and amplify it into numerous copies. Through the addition of primers, buffer, and thermal cycling, this technique denatures and anneals targeted DNA/RNA sequences to generate exponential copies. The reverse transcriptase polymerase chain reaction (RT-PCR) assay utilizes primers to qualitatively detect gene expression by transcribing messenger RNA in reverse to the complement DNA. Through the use of fluorescent probes, real-time PCR can quantitatively measure the amplification of DNA. The techniques of RT-PCR and real-time PCR can be combined for quantification of RNA. To measure the product of RT-PCR, samples are added to wells in agarose gel and separated with electrophoresis to determine if the gene is expressed based on molecular weight. With real-time PCR the samples are loaded into a plate with the probes and placed into the RT-PCR system. Measurement of sample fluorescence during thermal cycling is reported and the results are displayed as a graphical output.

The ability of PCR techniques to detect viral nucleic acid in small quantities during the febrile phase (i.e. before the formation of antibodies) is an advantage for early diagnosis (38). That ability to detect small quantities of the virus allows for the same methods to be applied to field samples (12). RT-PCR specifically offers rapid detection and high degrees of sensitivity and specificity for minute quantities of viral material. This makes it a useful technique with application to epidemiological studies, detection of virus in mosquito samples, and in clinical applications. Despite the number of advantages afforded by PCR, consideration must be given to the handling and storage of specimens as samples can be easily contaminated and RNA is easily destroyed by heat.

**Treatment and Prevention**

At this time there is not a licensed vaccine or other effective antiviral therapies for dengue (48). In addition, no specific treatment exists for dengue and patient care is dependent on clinical management of symptoms. The WHO treatment guidelines recognize a clinical continuum from dengue to severe dengue (56). Maintenance of the patient's body fluid volume is critical for severe dengue care. Mortality can be decreased by medical care from physicians and nurses experienced with the effects and progression of the disease (53). The only preventive measures to control dengue are limited to vector control and breaking human-vector interaction.

For vector control there are multiple methodologies and approaches that can be employed from the individual level all the way to the international organization level. Vector control includes chemical, environmental, and biological methods. The capacity of vectors to build up resistance, adapt to changes in their environment, and to be reintroduced into areas where they were once eradicated have led governments, institutions, and organizations to develop a systematic approach for vector control. In the United States this is called integrated pest management (IPM). The World Health Organization uses the term integrated vector management (IVM). At the core of both is a systematic approach to vector control. The WHO defines IVM as, "a rational decision-making process for the optimal use of resources for vector control" (56).

Chemical control measures for mosquitoes can target adult mosquitoes (adulticides) or immature forms (larvicides). Adulticides can be applied either as residual surface treatments or space treatments and can be utilized indoors or outdoors.

Organophosphates and pyrethroids are the two classes of adulticides used most widely today. Some applications of adulticides may also confer larvicidal effects.

Residual surface treatment applications are applied as a fine mist from a sprayer or mist blower with particles heavy enough to settle on and treat surfaces. Residual surface treatments provide lasting effects to kill adults that land or rest on surfaces. The WHO recommends indoor residual spraying (IRS) for malaria control since it is highly effective (1d). The main purpose of IRS is to reduce the transmission of disease by reducing the survival of endophilic vectors in homes (55). Roberts states that when IRS is used with a pesticide that has toxic, irritant, and repellent modes of action, then IRS is effective at killing susceptible vectors while still conferring a repellent action to deter the majority of resistant vectors (2). Given IRS in many instances may be used to specifically in malaria control, the fact that it functions on endophilic insects allows for it to contribute to the control of dengue vectors indoors.

Space treatments are commonly carried out through cold or thermal fogging. Cold fogging is a treatment applied as an aerosol from an ultra low volume (ULV) sprayer that utilizes pressure to produce microscopic droplets. Thermal fogging, on the other hand, is a treatment technique that utilizes heat to vaporize the chemical and dispense it in a fog. While both cold and thermal fogging offer quick knockdown of adult mosquitoes, both have a low residual.

Larvicides target the immature forms of mosquito and therefore are intended for the treatment of their aquatic habitat. The main compounds used as larvicides are organophosphates, insect growth regulators (IGR), biopesticides, and monomolecular films. Organophosphates act as neurotoxins to the immature mosquitoes. IGR prevent

immature mosquitoes from developing into adults. Biopesticides are microbial insecticides that interrupt absorption of nutrients in immature mosquitoes. Monomolecular films have a non-toxic mode of action that act to close off the respiratory structures of immature mosquitoes. Given the environment for treatment, the first consideration for the application of a treatment is whether the water is a domestic or drinking water source. As many *Aedes* breed in drinking water storage containers, it is important to consider the impact of larvicides on human health. It is also important to consider larvicides impact on nontarget species found living in conjunction with the vector species. The application of larvicides is dependent on the formulation. Liquid and wettable powders formulations can be sprayed while solid formulations can be applied by hand. Empty containers can be treated to prevent future larval infestations.

Environmental controls are preventive measures for the elimination of breeding sites and to reduce mosquito presence in areas near humans. Attention should focus on artificial containers that can hold or collect water. Items that are used to store or collect water should be covered to restrict access by ovipositing females. Animal watering containers or any other open containers like vases for flowers should be emptied and cleaned weekly.

The use of biological controls avoids the contamination of the environment with synthetic chemicals. These controls are based on organisms that feed upon, parasitize, or compete with mosquito larvae and adults to reduce populations. Examples of the organisms used for larval control include larvivorous fish species, *Toxorhynchites* larvae, and predatory freshwater crustaceans. Natural predators like bats, birds, reptiles, and insects are used in the control of adult mosquitoes by providing the appropriate habitat or

harborage for the predator. Consideration for the use of these controls must include the environment where they will be used and the cost.

With no existing treatment or immunization for dengue, preventive measures are necessary for residents exposed to *Aedes* mosquitoes. Use of personal protective measures to include N, N-Diethyl-meta-toluamide (DEET) on exposed skin and appropriate clothing are effective mosquito bite prevention measures (4). The use of window and door screens or the use of air conditioning can reduce mosquitoes indoors. Homeowners may also use household aerosol insecticides, mosquito coils, insecticide vaporizers or attractant traps to reduce mosquito biting (56).

### *AEDES AEGYPTI*

*Aedes aegypti*, known commonly as the yellow fever mosquito, is a vector for several arboviruses and diseases including DENV. *Ae. aegypti (s.l.)* is a species comprised of two subspecies, *Aedes aegypti formosus,* the ancestral sylvatic form found in sub-Saharan Africa and *Aedes aegypti aegypti,* found globally in association with humans in tropical and sub-tropical regions (41). *Aedes aegypti formosus* is a zoophilic, tree hole breeding species that is refractory to some endemic DENV serotypes to such a degree that it is not a significant DENV vector (48). *Aedes aegypti aegypti* is the subspecies that is referred to when discussing arboviral infections of humans involving *Aedes aegypti*.

The urban *Ae. aegypti* is a domestic species that exhibit both anthropophilic and endophilic behaviors and has adapted to the urban environment (15). Female mosquitoes are hematophagous, requiring a blood meal to complete the gonotrophic cycle. Females have developed specialized mouthparts for piercing while male mouthparts are not

developed for piercing. *Ae. aegypti* are primarily diurnal feeders with the typical peak feeding times of morning and late afternoon, though females can bite at night in areas that are well lit (5) Female *Ae. aegypti* mosquitoes approach from behind and feed around at the joints of lower extremities allowing it go unnoticed (5). Being nervous feeders, even slight movement will disrupt the feeding female. The female may return to the same human or different human moments later (10). During a single gonotrophic cycle a female is likely to feed up to three times before oviposition (11).

*Ae. aegypti* utilize a method of skip-oviposition where a few eggs are laid individually at several sites (26). The preferred oviposition sites are artificial containers with damp walls where single eggs are laid above the water line. The eggs can resist desiccation for several weeks or months in containers without water and then the larvae can hatch from the eggs when submerged in water. While the preferred sites of this adapted species are artificial containers such as flower vases, water storage containers, discarded tires, or other containers that may collect water, natural containers may be utilized (12). A key factor is that *Ae. aegypti* prefer clean water for oviposition over polluted sources. Some containers may only be seasonally productive when exposed to periods of rainfall where others may be productive throughout the year with a constant or near constant water supply.

Once the eggs have hatched the aquatic phase of the mosquito life cycle begins where the larvae will feed and develop through 4 instars, molting between each. From the fourth instar, the larvae will develop into pupae, a mobile, non-feeding transitional state, before emerging as an adult. Emergence of the adult ends the aquatic phase of the mosquito's life cycle. The process of larval-pupal ecdysis to emergence of an adult takes

about a week; however, it can vary with environmental factors of temperature, food availability, and habitat competition. After emergence, the adult life span mosquito may range from one week to a month, depending on gender and environmental conditions.

After emergence, a nectar meal is required for continued flight energy by both male and female mosquitoes. The innate ability of the *Ae. aegypti* for dispersion is a flight distance of about 30 to 50 meters a day which may limit the number of homes a female may visit in her life span though this may be modified by the availability of oviposition sites or food sources (12). In order to complete the reproductive cycle, male and female mosquitoes must mate. Females that mate with males will store sperm in the spermatheca for subsequent gonotrophic cycles. A mated female will seek out a blood meal in order to develop eggs. Once the female has taken a blood meal, she will rest for about two days before laying eggs and then go in search of another blood meal. Given the approximate life span of a couple weeks, the mosquito may take multiple blood meals to complete several gonotrophic cycles allowing an infected mosquito to transmit the virus. (5)

Adaptation of the *Ae. aegypti* to its environment makes it resilient to change with a rapid ability to rebound from natural disturbances or human interventions. The ability for the eggs to resist desiccation allows for population numbers to return to high levels once the eggs are exposed to favorable conditions (4). Though highly adapted to their urban environment, humans have significantly contributed to the success of the vector. Humans have provided passive dispersion through the transport of *Ae. aegypti* worldwide as well as providing suitable breeding sites within the domestic environment. Human homes provide ideal shelter for *Ae. aegypti* harborage as they prefer to rest in darker

cooler areas (4). This harborage is collocated with larval habitat and the preferred food source (15) which leads to *Ae. aegypti* biting indoors (4). In areas with poverty, *Ae. aegypti* may be supported by the lack of municipal water sources that require domestic water storage, as well as, the lack of refuse collection which may result in water holding waste receptacles that support larval development (48).

### *AEDES AEGYPTI* SURVEILLANCE & SAMPLING

Surveillance for the disease and the vector is important to the prevention and control of dengue. Disease surveillance is a systematic process reflective of the health status of a population in order to prevent or control a disease (56). Vector surveillance consists of measurements of the vector population. Vector measurements can be made temporally and spatially. Surveillance for dengue vectors tends to focus on all mosquito life stages.

The urban *Ae. aegypti* populations provide a challenge to adult surveillance. According to Gubler (12), adult populations of *Ae. aegypti* are not easy to estimate and surveillance is labor intensive. When choosing a sampling method, the first two considerations should be the purpose for the survey and the scope of the survey area (37). The types of adult collections can be divided into landing collections, resting collections, and trap collections. Landing collections utilize humans as bait and mosquitoes are captured using aspirators or nets. This method is generally not employed with dengue surveillance due to the potential for transmission of virus to a naive collector. Resting collections target indoor mosquitoes during periods of inactivity using some form of aspiration device. Trapping consists of using some form of attractant (visual or olfactory) to lure mosquitoes to a collection device. When sampling adult *Ae. aegypti*, there are

currently two commonly used methods; Prokopack aspiration and the BioGents-Sentinel™ (BGS) trap.

Gonzalo Vazquez-Prokopec et al. (47) reported the development of a new mosquito aspirator with performance akin to the CDC Backpack Aspirator (CDC-BP), which they called the Prokopack. While the CDC-BP can be considered the "gold standard" sampling tool for adult *Ae. aegypti* (50), it is expensive (US $468 – 758). The CDC-BP is limited by its weight and non-extendable rigid suction that restricts its reach. The Prokopack maintains a lighter weight, lower cost, and an extendable arm making it an effective alternative to the CDC-BP. (47).

The BioGents-Sentinel™ (BGS) (Biogents AG, Regensburg, Germany) trap became available in 2006. It is an effective *Ae. aegypti* sampling device which is capable of collecting more females than the CDC-BP while offering a more standardized collection method (50). Collection data from BGS traps can be used to estimate dispersal, survival, and parity rate of mosquito populations (20). Since captured females remain alive, viruses may be isolated from them. This allows the monitoring of dengue virus circulation leading to better characterization of dengue transmission in a given area (20). The BGS may be more useful than aspirator type sampling devices if house entry is problematic due to noncompliance by residents or for cultural reasons as it can be set in a sheltered area immediately outside the house (51).

Larval surveillance consists of surveying containers for the presence of larvae. Three common larval surveys are (12):

House index: Number of houses positive for *Ae. aegypti* larvae and/or pupae per 100 houses.

Container index: Number of habitats positive for *Ae. aegypti* larvae and/or pupae per 100 potential habitats.

Breteau location index: Number of habitats positive for *Ae. aegypti* larvae and/or pupae per 100 locations.

These indices are measures of positive containers for *Ae. aegypti* larvae but are not intended to measure the actual number of larvae present at a location. These indices also do not quantify the productivity or the classification of the container. In order to conduct these surveys a systematic search of the location must be conducted and all containers must be observed for the presence of larvae. These indices do not address several factors but still tend to be the most widely used (35). Due to the biological factors that affect productivity of different containers, larval indices are a poor indication of adult production (56).

Pupal surveillance, likewise, targets container habitats used for mosquito oviposition and immature development. The purpose of this technique is to estimate the number of adults per container based on the correlation to pupal density (3). Focks (9) proposed a pupal-demographic survey method as a means of determining transmission thresholds based on the adult to pupal correlation and human density (9). During the collection and counting of pupae, the containers can be evaluated for production. When

the highest producing containers are known they can be targeted for source reduction. As these surveys may be too labor intensive they are not conducive for routine monitoring purposes (56).

Oviposition traps, or ovitraps, function on the nature of the *Aedes* mosquitoes to lay their eggs above the water line of a container. Typical ovitrap construction consists of a black glass or plastic cup partially filled with water and a wooden paddle or paper strip where the mosquitoes can lay their eggs (12). Ovitraps have been adapted through several modifications that include the addition of an organic infusion in the water to increase attractiveness, the addition of sticky strips to trap landing females, and autocidal traps that either mechanically trap adults and larvae or poisons them (37). While ovitraps offer a sensitive measure of the presence or absence and are useful for observing spatial and temporal distribution, comparisons between areas at the same point in time cannot be made reliably (9).

### GEOGRAPHIC INFORMATION SYSTEM

A geographic information system (GIS), according to Sabins (33), "is an organized collection of computer hardware and software, with supporting data and personnel, that captures, stores, manipulates, analyzes and displays all forms of geographically referenced information." A GIS system links attribute data to spatial data into layers for analysis and display. Often GIS is used with processed remote sensing images and data that are acquired from aircraft or satellites with active and passive sensors. With the availability of remote sensing data, inexpensive global positioning systems, and the use of other georeferenced data, GIS has the strengthened potential for use in disease epidemiology and vector control (37).

The scale on which the system and spatial data is employed is essential to GIS use for dengue and vector surveillance and analysis. Scott and Morrison (36) found that frequent measurements, at the household level, are best for determining entomological risk for dengue noting that risk prediction can vary across different geographic scales. In developing two complementary simulation models for urban dengue fever, one an entomologic model and the other a transmission model, Focks et al. (8) found that while these models provide estimates, they are only useful for the locale that they were developed for. Understanding these limitations and how they relate to disease transmission, use of GIS as a quantitative assessment of entomological and epidemiological data, should be considered as a decision making tool for vector and dengue control (25).

**SINT EUSTATIUS AND DENGUE**

Sint Eustatius, known as Statia, located at 17°29′N 62°59′W shown in Figure 1, was once part of the Netherland Antilles and part of the Kingdom of the Netherlands. The Netherland Antilles consisted of two island groups in the Caribbean Sea, composed of five islands including Curaçao and Bonaire, located off the coast of Venezuela, and Sint Maarten, Saba, and Sint Eustatius, lying to the southeast of the US Virgin Islands. The official language of the Netherland Antilles is Dutch though English is widely spoken as a first language. Since October 10, 2010, St. Eustatius has been a member of the BES islands (Bonaire, Eustatius and Saba) and part of the Caribbean Netherlands (40). St. Eustatius is located in the northern Leeward Islands portion of the West Indies and forms part of the inner arc of the Leeward Island chain, lying immediately to the

northwest of Saint Kitts and Nevis and to the southeast of Saba. Sint Eustatius has a land area of 21 km$^2$ (27).

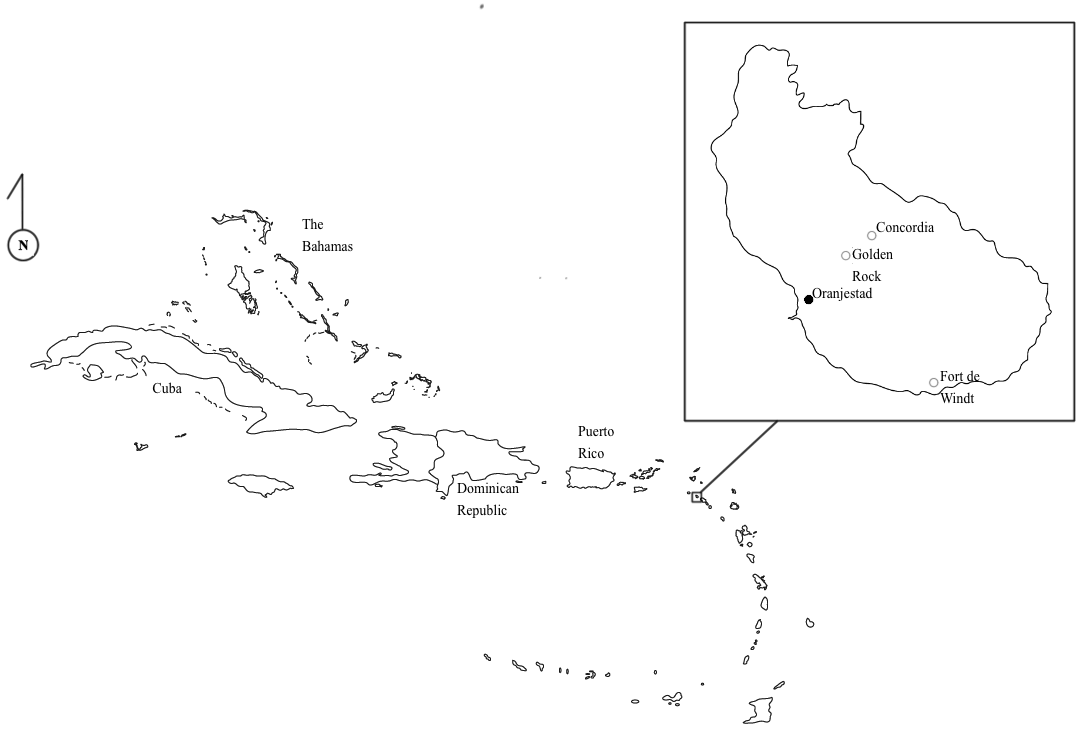


**Figure 1.** Map of Sint Eustatius Netherland Antilles and Caribbean.

Christopher Columbus first sighted Sint Eustatius in 1493. During the colonial era the island changed hands many times and in 1636, with the close of an 80-year war between the Netherlands and Spain, the Dutch claimed possession. During the 1600's through the 1700's, the island was a major trading center with one of the busiest ports in the Caribbean where thousands of ships were sailing to its shores and 20,000 inhabitants. During the American Revolution, Sint Eustatius was an important shipping port to the thirteen colonies with open trade and movement of arms and ammunition from France, providing prosperity for the island as the United States fought for independence (17).

On November 16, 1776, Sint Eustatius was the first foreign nation to officially recognize the newly formed United States of America. After the end of the American Revolution, St. Eustatius was the leading trade island in the Caribbean where thousands of ships anchored at Oranjestad and the shore was lined with warehouses packed with goods. It reached its economic peak around 1795 (40). With the passing of the eighteenth century St. Eustatius lost its importance as a trading center and with that trading importance gone most merchants and planters left the island. It was in the nineteenth century that Sint Eustatius settled in being a quiet island.

In 2010, the population of Sint Eustatius was reported as 2,886 (27). The majority of the native population is of African and mixed African descent. The immigrant population is comprised of individuals from other Caribbean islands, China, Europe, and North America.

There were 22 confirmed cases of dengue in 2010 though no confirmed reports of dengue hemorrhagic fever on the island (27). There are three physicians on the island and a 20 bed general hospital (27). The government is the largest employer (39). Currently there is one public health department employee who conducts mosquito vector surveillance and control in addition to other duties. The regularly employed mosquito control measures consist of treating larval habitat with larvicide.

**PREVIOUS STUDIES**

From 1948 to 1954, van der Kuyp (16, 17, & 18) had three publications from an entomological survey of adult and immature mosquitoes in April of 1947. Species found during this survey include: *Aedes aegypti (*Linnaeus*), Aedes busckii* (Coquillett), *Culex quinquefasciatus* (Say), *Culex americanus* (Neveu-Lemaire), and *Culex bahamensis*

(Dyar & Knab) (Fig. 2). Van der Kuyp noted that it was an unfavorable season for conducting a mosquito survey, as it was the dry season (16). At this time there was a population of 970 inhabitants with only one physician. Larval habitats were identified as barrels, drums, wooden tubs, troughs, stoneware jars, and earthen water pitcher, and concrete blocks for *Ae. aegypti*. Of the 31 yards examined for *Ae. aegypti*, 24 were found infested yielding a 77.4% infestation rate (18). Van der Kuyp stated that at that time there was little evidence of the presence of mosquito-borne diseases though older inhabitants recalled epidemic tertian fever at the turn of the century (17).


**Figure 2.** Reproduction of collection sites for April 1947 van der Kuyp survey (11).

An *Ae. aegypti* eradication campaign was conducted in 1951 with assistance from PAHO/WHO that led to a temporary absence of this species until 1954. The lack of campaign maintenance led to its reintroduction and a renewed eradication program in 1974/1975 (34). Knudsen (14) indicated the presence of *Ae. aegypti* on Sint Eustatius in an overview published on the vector and dengue in the Caribbean. The method of sampling or a source for documentation of the presence of the species was not indicated.

From May - September 1972, a serological survey was conducted to consider the status of dengue immunity of school age children in Sint Eustatius following the endemic dengue activity that had been recorded on the island in a 1970 all age study (34). Sera were obtained from 309 school children (5 – 16 years old), and tested for hemagglutination inhibiting (HI) antibodies with group A and group B arbovirus antigens, Togaviridae and Flaviviridae respectively. Results for the HI testing found only group B antibodies of DENV 1, DENV 2, and DENV 3 with 63% of the 309 sera being positive and DENV 2 having the highest frequency (34). Included with these results were the findings of the all-age serology survey which showed 94% DENV positive with the frequency of DENV 2 > DENV 1, except for the 60 – 69 age group that had DENV 1 > DENV 2, a low frequency of DENV 3 and findings consistent with endemic DENV 2 (34). Van der Sar stated that the *Ae. aegypti* eradication campaign did not reflect a "favorable result" in his data (34). The results of the Van der Sar serology survey are summarized in Tables 1-4.

**Table 1.** School age children with antibodies to group B arboviruses in Sint Eustatius from 1979 Van der Sar serology survey (34).

| *Age* | *5* | *6* | *7* | *8* | *9* | *10* | *11* | *12* | *13* | *14* | *15* | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | 20 | 38 | 32 | 43 | 36 | 44 | 25 | 30 | 20 | 21 | No data | 309 |
| % pos | 55 | 47 | 34 | 41 | 55 | 77 | 64 | 90 | 90 | 86 | No data | 63 |

**Table 2.** School age children with HI antibodies for Dengue in Sint Eustatius from 1979 Van der Sar serology survey (34).

| | *Dengue 1* | *Dengue 2* | *Dengue 3* | *Dengue 4* |
|---|---|---|---|---|
| No. | 157 | 185 | 96 | 0 |
| % pos | 51 | 60 | 31 | 0 |

**Table 3.** All ages survey with antibodies to group B arboviruses in Sint Eustatius from 1972 Van der Sar serology survey (34).

| Age group (years) | 0-9 | 10-19 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70-79 | 80+ | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| No. | 159 | 211 | 105 | 96 | 75 | 82 | 94 | 29 | 18 | 869 |
| % pos. | 86 | 82 | 88 | 85 | 91 | 98 | 96 | 100 | 100 | 91 |

**Table 4.** Sint Eustatius activity of dengue types according to Age Group from Van der Sar 1979 (34).

| Age Group (Years) | 0-19 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70+ |
|---|---|---|---|---|---|---|---|
| D1>D2 | 5 | 9 | 3 | 15 | 8 | 29 | 6 |
| D2>D1 | 30 | 16 | 21 | 23 | 25 | 16 | 26 |
| D1=D2 | 61 | 75 | 75 | 62 | 68 | 54 | 69 |

Most recently, a dengue serosurvey was conducted in the fall of 2012 in Sint Eustatius. Researchers from the University of Maryland and Naval Medical Research Center collected 3 ml blood serum samples from 177 participants in this cross sectional all age survey (19). The serosurvey utilized ELISA and $PRNT_{50}$ for flavivirus and DENV detection. From the analyzed sera, 88% had antibodies for flaviviruses and 83% of these were positive for one or more DENV serotype (19). The study indicated that DENV 2 is the most prevalent on the island and is the only serotype present in the 10 – 19 age group (19).

# CHAPTER 2: Purpose of This Study

## STUDY AIMS

### Hypothesis:

Spatial differences in Sint Eustatius are associated with the number and distribution of *Aedes* larval breeding sites and thereby impact the distribution of adult *Aedes* mosquitoes and dengue infections.

#### Specific Aim 1 (Sampling Technique Comparison):

Examine the sampling efficiency of indoor Prokopack aspiration of *Ae. aegypti* as compared to exterior trapping with the BioGents-Sentinel™ (BGS) Trap.

##### Objective 1:

Evaluate the effectiveness of both the BGS and indoor Prokopack as effective tools for *Ae. aegypti* surveillance.

##### Objective 2:

Assess the use of the BGS in the peridomestic environment as an effective tool for estimating indoor *Ae. aegypti* densities (in the instance that indoor access is restricted or denied)

#### Specific Aim 2 (Domestic Survey):

Characterize the spatial distribution of larval and adult *Aedes aegypti* populations and determine risk factors associated with dengue positive mosquito survey data, remote sensing data, and dengue virus detection data.

**Objective 1:**

Characterize the domestic and peridomestic risk factors associated with high densities of larval and adult *Ae. aegypti* using collected data on container attributes, household description, and environmental factors.

**Objective 2:**

Examine the relationship between *Ae. aegypti* positive larval breeding sites, sampled adults, and dengue distribution.

**Objective 3:**

Develop risk maps from acquired larval, adult and dengue positive mosquito pools for the study area.

# CHAPTER 3: Materials & Methods

## STUDY DESIGN

Prior to this study, the Uniformed Services University of the Health Sciences and the University of Maryland Institutional Review Boards approved the protocol for this study. This study was conducted for six weeks July - August 2012. An open enrollment was conducted to solicit volunteer households for the study. Prior to the enrollment, flyers were posted and radio advertisements sponsored by the Department of Health were used to advertise the study. There were a limited number of volunteers and in order to facilitate the study, recruitment was conducted door to door from 0800 until 1700hrs concurrent with sampling. An objective of ten houses in each neighborhood was set in order to obtain a representative sample across all homes.

Before entering a household, verbal consent was obtained from an adult resident of the home. In obtaining the verbal consent, a written script was used to explain the sampling process, the equipment and how it functioned, how the data would be used, and who the members of the survey team were and their respective organization affiliation. House locations were recorded with a Garmin GPSmap 60CSx (Garmin Ltd., Schaffhausen, Germany) with 3-meter accuracy or less, where the drive or walk connected to the street for all homes visited. Houses were given an identification number based on their neighborhood and the total number of houses surveyed. This house number served as the base for the identification numbers for samples obtained from the house or premises in order to link the data. The house number and coordinates were

recorded in a logbook and recorded on data sheets corresponding with the sample. With consent, the survey team split into two groups that sampled either indoors or outdoors.

Outdoors sampling: The survey consisted of recording house characteristics and surveying for larval habitats. In addition to the structural characteristics of the house (see Appendix 1), data were recorded on the types and number of animals present, distance to the nearest house, water source, and refuse water disposal. For the larval survey, all containers or structures that could hold enough water to support immature development were observed for immature stages. When immature stages were present, the container was identified as "positive." Identified positive containers were given an identification number, which was then recorded on a survey sheet (see Appendix 3) along with the characteristics for each positive container. Recorded characteristics included the container type, volume, construction, and container opening height above ground, distance from house, shade, and aquatic vegetation. Metrics for each of these characteristics were included in the survey sheet in order to provide discrete classification of each characteristic. In addition, data were recorded on precipitation, vegetation in the vicinity, and surrounding terrain.

Immature mosquitoes were then sampled from all mosquito positive containers on the premises. Small containers, less than 25 liters and capable of being moved, were emptied into a white plastic lab pan. Containers that could not be moved or had a volume greater than 25 liters were sampled using a plastic kitchen baster. The water from the baster was then transferred to a white plastic lab pan. Immature forms were then removed using a plastic dropper with a suction bulb to Nasco Whirl-Paks®, labeled with the identification number and date, for storage until processing. Sealed Whirl-Paks®

were placed in a container of water to limit disturbance of the water surface inside the bag to reduce possible mortality in the samples during transport.

Samples in Whirl-Paks® were transferred to 1 pint plastic ice cream cups labeled with identification number and date. Ice cream cups were filled to approximately one-third the volume with fresh water and two or three flakes of Tetramin (Tetra Werke, Melle, Germany) fish food were added. A cloth mesh screen was placed over the top of the cup to eliminate the possibility of a newly emerged adult escaping and to eliminate possible contamination of the sample through oviposition of females free in the environment. The sample cups were checked daily for the presence of new pupae and to monitor water level. Pupae were removed and transferred to a second cup marked and filled in the same manner, covered with mesh and the adults were allowed to emerge. Once all mosquitoes had emerged, the entire cup was placed in a freezer to kill the mosquitoes.

Killed mosquitoes were placed in plastic petri dishes and sorted under a dissecting microscope by species and sex. Mosquitoes were identified using *Pictorial Keys for the Identification of Mosquitoes (Diptera: Culicidae) Associated with Dengue Virus Transmission* (32). The sex and species identification was recorded on the same survey sheet that was used at the time of sampling (see Appendix 2). After recording data, processed samples were discarded.

Indoors sampling: Prokopacks were used for mechanical aspiration of mosquitoes. Prokopacks were assembled as described by Vazquez-Prokopec et al. (47). In the construction, a lighter extendable aluminum pole was substituted for the larger painting extension pole described. A total of 3 Prokopacks were constructed and labeled as 1, 2,

and 3. Power was provided to the Prokopacks through 12 volt DC 10 amp hour maintenance free battery. A total of 4 batteries, labeled as 1, 2, 3, and 4, were used in rotation. Batteries were charged for a minimum of 8 hours after being used for 3 iterations of sampling. Each battery was used no more than three times in a given day. The 14 screened collection cups used by the Prokopack were also numbered. At the time of the sample, an identification number and the date time group were recorded on a piece of tape placed on the cup. On the data sheet (see Appendix 2) corresponding to the Prokopack sample, the identification number of the Prokopack, battery, and cup used were recorded.

The indoor sampling was conducted for 15 minutes in each sampled house. During the sampling period a single operator conducted sampling throughout the house in all rooms that the resident allowed access. The method of sampling consisted of continuous aspiration along the border of internal wall junction with the floor and ceiling, behind doors, and around moldings, trim, and framing in addition to under, inside, and behind furniture, fixtures, cabinetry, shelves, and closets where accessible to target mosquito resting sites. The start and end time, total number of rooms, number of rooms sampled were recorded on the data sheet (see Appendix 2). The resident provided information on the number of occupants, insecticide usage, and the general time that windows and doors were open. Captured adults were held in the screened collection cups until processing.

The collection cups were placed in a freezer in order to kill the mosquitoes. Killed mosquitoes were transferred to a plastic petri dish on a chill table. With the aid of a dissection microscope (10X), they were sorted by species and sex. Mosquitoes were

identified using *Pictorial Keys for the Identification of Mosquitoes (Diptera: Culicidae) Associated with Dengue Virus Transmission* (32). Data on gender and species were recorded on the same survey sheet that was used at the time of sampling (see Appendix 2). Identified samples were then placed in 1.5 ml cryogenic vials labeled with the sample identification number, total, gender, and species. If genera other than *Aedes* were collected, they were not identified to species but the genera were recorded. Sealed vials were then placed in a dry shipper, charged to -80 ºC for storage. Samples were cold shipped to the Naval Medical Research Center (NMRC) Silver Spring, MD for analysis.

Fourteen houses from the general survey that were mosquito positive were selected for inclusion in the comparative sampling study. These houses were sampled by Prokopack for longitudinal survey data. A minimum of 7 days was allowed from the time of the initial Prokopack sampling from an initial survey of 81 homes until the second indoor Prokopack sampling session for this comparative study. The second sampling session was conducted by unit area time, 1 minute per 100sq feet. Other than the change to the sampling duration, the methodology remained unchanged. This sample was given a subsequent identification number and the same metrics were recorded on a data sheet (see Appendix 2).

The outdoor sampling of adult mosquitoes was conducted by trapping in the peridomestic environment. The BGS traps were assembled in accordance with the provided manufacturer instructions and were set in areas sheltered from wind, direct sunlight, rain, and as close as possible to an entryway or window to the house being sampled. Outdoor trapping was divided into a morning and an evening period that was matched to the time of indoor sampling. Three traps, marked 1, 2, and 3, were used in a

random order at target locations. A single trap baited with an olfactory attractant was placed at each household. The BGS traps were powered by the same 12 volt DC 10 amp hour maintenance free batteries that were used for Prokopack sampling. Once placed, the traps were allowed to run continuously for 4 hours. A 1" x ½" piece of paper with the sample identification number and date, time and group was used to verify the correct flow of the trap and then dropped into the catch bag. The trap number, battery number, and sample identification were recorded on a data sheet (see Appendix 4) in addition to start and end time for the collection.

At the end of the trapping period, the catch bags were removed, sealed, and transported for processing. The catch bags were immediately placed in a standard kitchen freezer upon arrival at the field lab in order to kill the mosquitoes. Killed mosquitoes were transferred to a plastic petri dish on a portable chill table and sorted with the aid of a binocular dissection microscope (10X) by species and sex. Mosquitoes were identified using *Pictorial Keys for the Identification of Mosquitoes (Diptera: Culicidae) Associated with Dengue Virus Transmission* (32). The data on gender and species were recorded on the same survey sheet that was used at the time of sampling (see Appendix 4).

The homes surveyed during the general survey were used for the characterization of the spatial distribution of larval and adult *Aedes aegypti* populations and determination of risk factors associated with dengue positive mosquito pools. From the general survey a subset of 11 homes located in the Golden Rock Neighborhood. These houses were characterized as government housing and were roughly identical in construction, yard size, and distance to nearest home to each other. This cluster of homes

was compared to a second cluster of 72 houses from the general survey that was considered heterogeneous.

### LABORATORY PROCESSING

In the NMRC Viral and Rickettsial Disease Department (VRDD) bio-safety level 2 laboratory, mosquito samples were analyzed for the presence of dengue virus. Upon receipt of the frozen samples, the inventory was entered into the VRDD database for storage management and tracking. Mosquitoes were transferred from the 1.5 ml cryovials used for shipping and storage to 1.5 ml conical tubes that were sample identification number labeled. As the mosquitoes were transferred, the hind legs were removed and placed in a single 1.5 ml cryovial, which was provided to the Walter Reed Biosystematics Unit for their mosquito barcoding initiative. For sample sizes greater than 10, the samples were divided equally into the number of conical tubes required to have a pool size of less than 10 mosquitoes. Before homogenization, 200 $\mu$l of cell culture medium was pipetted into the vial. Samples were homogenized using disposable plastic pestles with a portable tissue grinder inside a biological safety cabinet.

Aliquots of each sample were transferred 1.5 ml conical tubes for polymerase chain reaction detection of dengue virus. RNA was extracted from the samples using QIAmp Viral RNA kit (Qaigen, Venlo, Netherlands) following the VRDD standard operating procedures for this kit. The extracted RNA template was analyzed in an Applied BioSystems TaqMan PCR (Life Technologies, Grand Island, NY) system using an Invitrogen Express One-Step SuperScript qRT-PCR Kit (Life Technologies, Grand Island, NY) following the VRDD standard operating procedures. This was a real-time PCR (qPCR) analysis utilizing McAvin Primers. The qPCR cycling conditions are 50° C

for 15 min hold, 95° C for 2 min hold, 40 cycles of 95° Cfor 15 sec and 60° C for 1 min, and a 4° C hold following amplification.

### ANALYSIS

Statistical analysis of samples was conducted using IBM SPSS Statistics version 20 (IBM Corporation, Armonk, New York). Comparison of Prokopack and BGS methods were analyzed with descriptive statistics, t-tests and multiple linear regressions. Sampled densities weree Log10 (x+1) transformed to meet normality requirements. A separate analysis of house characteristics, breeding container characteristics, human density, in addition to adult and larval densities were analyzed through descriptive statistics, t-tests and multiple linear regressions to compare sampled areas and evaluate habitat and container characteristics.

Analysis of the geographic of *Ae. aegypti* distribution was conducted with ArcGIS version 10 (ESRI, Redlands, California). Analysis utilized known variables, sampled densities, and limits of *Ae. aegypti* habitat. Distribution of sampled mosquitoes were compared to digital elevation model (DEM) data using ArcGIS extract point feature and statistics to analyze the mean elevation data. The DEM data was from the Shuttle Radar Topography Mission (SRTM) originally produced by NASA and now available for download from the Consortium for Spatial Information (CGIAR) at a 90 meter resolution.

Collection sites were mapped according to density classification. Inverse distance weighting (IDW) using the Geospatial Analyst Tools in ArcGIS were used to interpolate density data for adult female *Ae. aegypti* and containers positive for *Ae. aegypti* larvae. The IDW function uses measured values to predict surrounding values for any

unmeasured location. IDW is based on the explicit assumption that objects that are close together are more similar than those far apart. The IDW projection was added as a layer to the collection data to create a map of observed and predicted values. Maps created from this analysis in the future could be used to develop sampling strategies and possible vector controls programs for Sint Eustatius.

# CHAPTER 4: Sampling Technique Comparison

## STATISTICAL RESULTS

A total of 172 adult *Ae. aegypti* were sampled from the 14 homes (Fig. 3) for the examination of sampling efficiency of the Prokopack aspirator and BGS traps for *Ae. aegypti*. From the 14 homes, aspiration indoors with the Prokopack captured 77 *Ae. aegypti* (44.8%), while outdoor trapping with the BGS captured 95 Ae. aegypti (55.2%). Comparison of means with independent samples t-test, equal variances not assumed, showed no significant difference ($t_{21} = 0.44$, $p = 0.66$) in Prokopack aspiration and BGS trapping at the $\alpha = 0.05$ level of significance. In total, the mean number of *Ae. aegypti* collected was 5.50 ± 0.95 SE for the Prokopack and 6.79 ± 2.00 SE for the BGS with Log10 (x+1) values being 0.74 ± 0.07 SE and 0.68 ± 0.12 SE respectively. There was no significant difference in the total number of *Ae. aegypti* collected by Prokopack as compared to the BGS ($t_{21}= 0.52$, $p= 0.66$). Prokopack aspiration collected 50.7 *Ae. aegypti*/man hour while the BGS collected 1.7 *Ae. aegypti*/trap hour for the 14 homes in this comparative study. The results are summarized in Table 5.

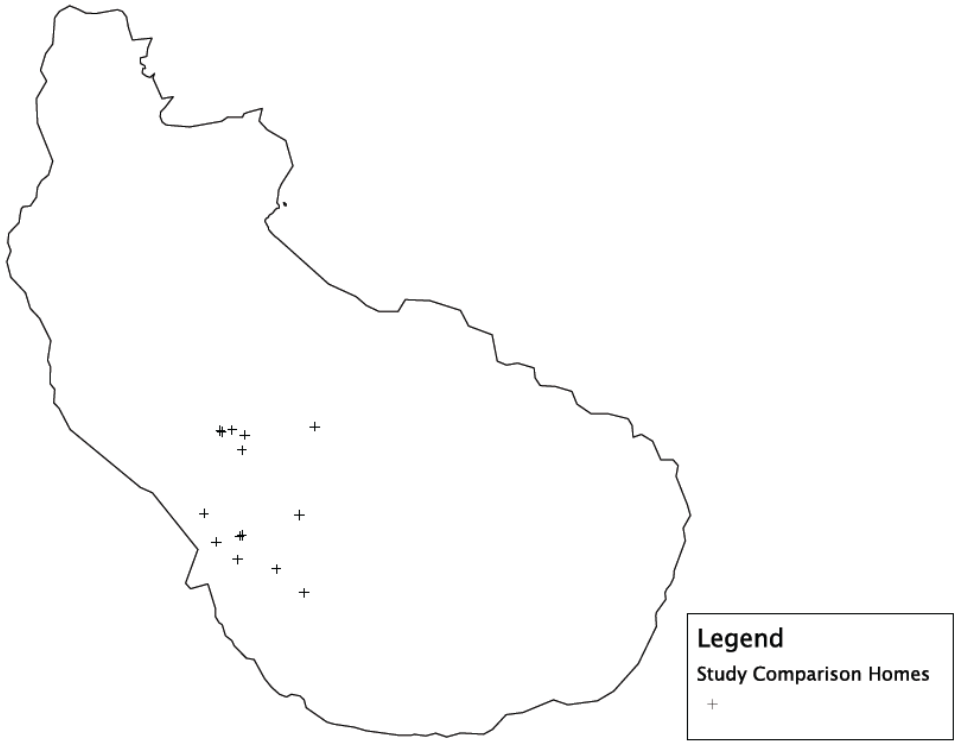


**Figure 3.** The location of the 14 homes on Sint Eustatius sampled for the comparison of Prokopack and BGS.

**Table 5.** *Ae. aegypti* adult collection data gathered using two sampling methods in Sint Eustatius.

| | Prokopack[1] | BG-Sentinel Trap[2] | Statistical Result[3] |
|---|---|---|---|
| **Total *Ae. aegypti*** | 77 (44.8%) | 95 (55.2%) | $t_{21} = 0.44$ $p = 0.66$ |
| ***Ae. aegypti* ♀ : ♂** | 40 : 37 | 53 : 42 | |
| **Mean[2] ♀ *Ae. aegypti*** | 2.86 ± 0.46 | 3.79 ± 1.00 | |
| Log10(x+1) | 0.543 ± 0.06 | 0.540 ± 0.10 | $t_{20} = 0.03$ $p = 0.98$ |
| **Mean[2] ♂ *Ae. aegypti*** | 2.64 ± 0.68 | 3.00 ± 1.25 | |
| Log10(x+1) | 0.466 ± 0.08 | 0.394 ± 0.11 | $t_{24} = 0.52$ $p = 0.61$ |
| **Mean[2] *Ae. aegypti*** | 5.50 ± 0.95 | 6.79 ± 2.00 | |
| Log10(x+1) | 0.744 ± 0.07 | 0.681 ± 0.12 | $t_{21} = 0.52$ $p = 0.66$ |
| **Collection Rate** | 50.7 *Ae. aegypti*/ Man Hr | 1.7 *Ae. aegypti*/ Trap Hr | |

[1] Indoor aspiration collection

[2] Outdoor scent baited trap

[3] Independent samples t-test with equal variances not assumed at the $\alpha = 0.05$ level of significance.

[4] Means ± Std Error

A total of 93 adult female *Ae. aegypti* (54.1%) were sampled during the comparative study with 40 (43.0%) coming from the Prokopack aspiration and 53 (57.0%) coming from the BGS trapping. The mean number of female *Ae. aegypti* collected was 2.86 ± 0.46 Standard Error (SE) with the Prokopack and 3.79 ± 1.00 SE with the BGS. These values were Log10 (x+1) for normality resulting in 0.543 ± 0.06 SE for the Prokopack and 0.540 ± 0.10 SE for the BGS. There was no significant difference in the numbers of female *Ae. aegypti* collected by Prokopack versus BGS ($t_{20}$= 0.03, p= 0.98) at the $\alpha = 0.05$ level of significance.

A total of 79 (45.9%) male *Ae. aegypti* were sampled during the study with 37 (46.8%) from Prokopack aspiration and 42 (53.2%) from BGS trapping. The mean number of male *Ae. aegypti* collected was 2.64 ± 0.68 SE with the Prokopack and 3.00 ± 1.25 SE with the BGS with Log10 (x+1) values being 0.47 ± 0.08 SE and 0.39 ± 0.11 SE respectively. Again, there was no significant difference in the number of male *Ae. aegypti* collected by Prokopack as compared to the BGS ($t_{24}$= 0.52, p= 0.61) at the $\alpha$ = 0.05 level of significance.

**CORRELATIONS**

To model the relationship of indoor Prokopack aspiration, outdoor BGS trapping and sampling of larval positive containers, linear regressions were performed on Log10 (x+1) transformed values of the three sampling methods. The use of Log10 (x+1) transformed values was to meet data distribution normality requirements for statistical analysis. There was a positive linear correlation ($R^2$= 0.35) between the numbers of *Ae. aegypti* obtained from the BGS collection outdoors as compared to the numbers of *Ae. aegypti* collected indoors by Prokopack aspiration. These variables had a statistically

significant ($t_{12}$= 2.55, p= 0.03) linear relationship at the $\alpha = 0.05$ level of significance indicating outdoor trapping was useful as a predictor of indoor sampled densities (Fig. 4).

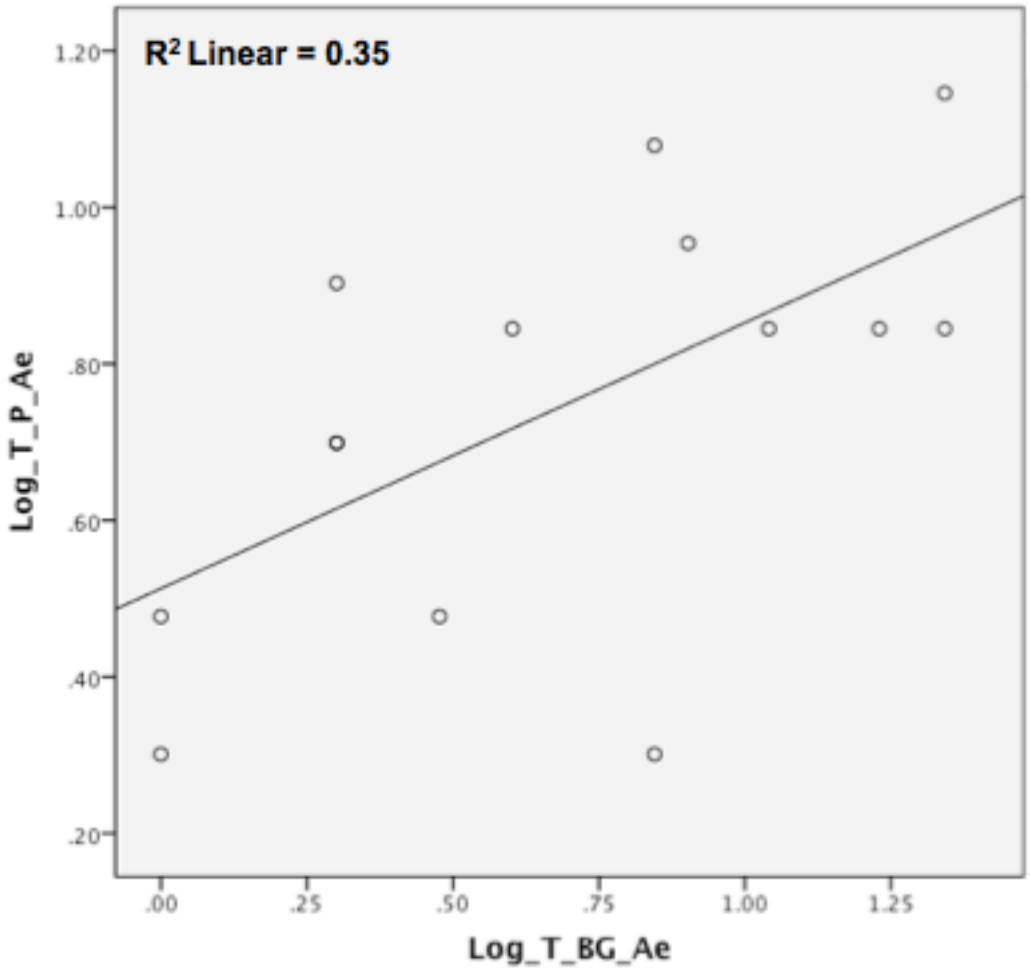


**Figure 4.** Sampled Prokopack densities versus sampled BGS densities.

Examination of these data points revealed that one sampled home had a marked decrease in the number of *Ae. aegypti* sampled indoors. During the first survey of this home there were 75 (18♀ and 47♂) *Ae. aegypti* present while during the standardized 100 square feet per 1 minute there was only 1♀*Ae. aegypti* present. The reason for this 75 fold decrease indoors cannot be explained as there was not a visible change in the home environment at time of the standardized sampling. Removing this data point from

the linear regression analysis resulted in a change in the linear correlation ($R^2$= 0.54) and statistical significance ($t_{11}$= 3.60, p= 0.01) at a $\alpha$ = 0.05 level of significance strengthening the positive linear relationship (Figure 5).

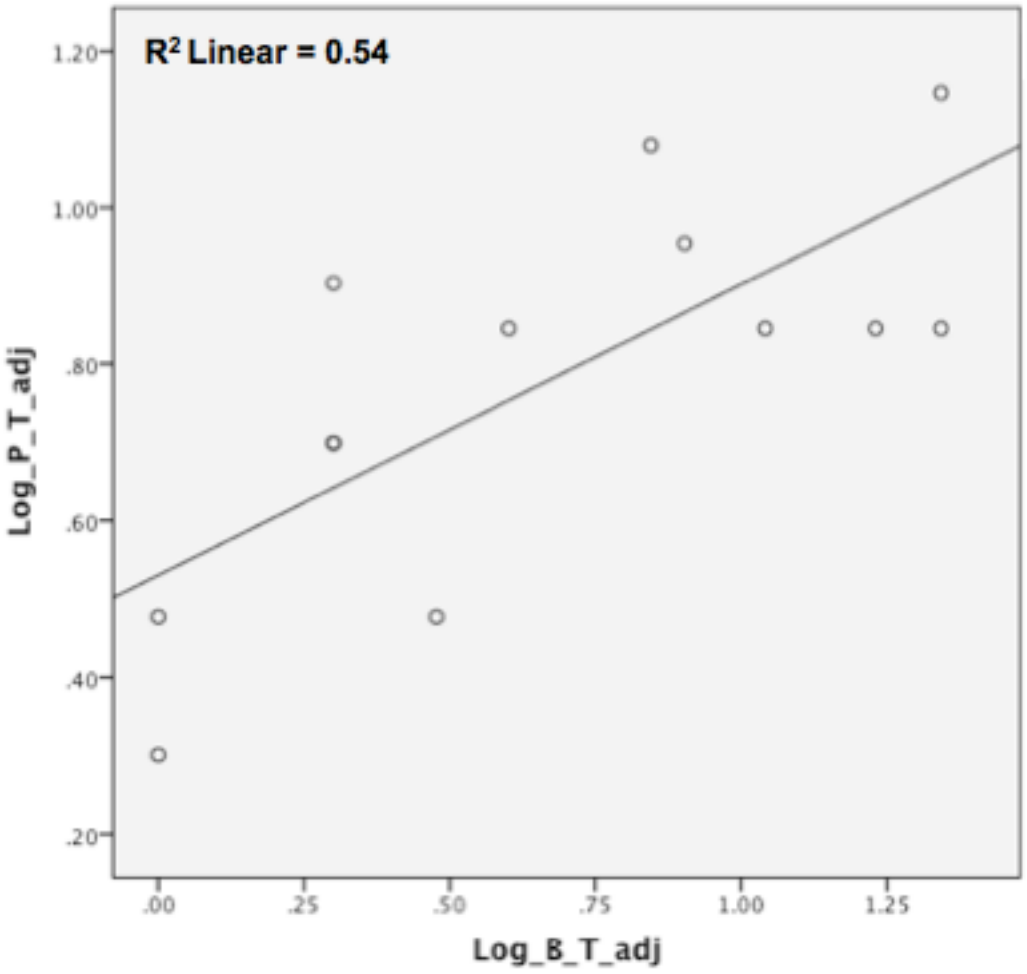


**Figure 5.** Sampled Prokopack densities versus sampled BGS densities with censored point.

When outdoor larval densities were used as a predictor of indoor Prokopack densities, there was a weak linear correlation ($R^2$= 0.03). At the $\alpha$ = 0.05 level of

significance there was not enough evidence ($t_{12}$= -0.64, p= 0.54) to suggest that the slope of the regression line was different than zero. This suggested that larval densities could not be used for predicting indoor Prokopack densities (Figure 6). However, removing the outlier using the explanation as in the previous evaluation, the new linear regression analysis resulted in a significant positive linear correlation ($R^2$= 0.01) ($t_{11}$= -0.30, p= 0.77) at a $\alpha = 0.05$ level of significance strengthening the positive linear relationship (Fig. 7).

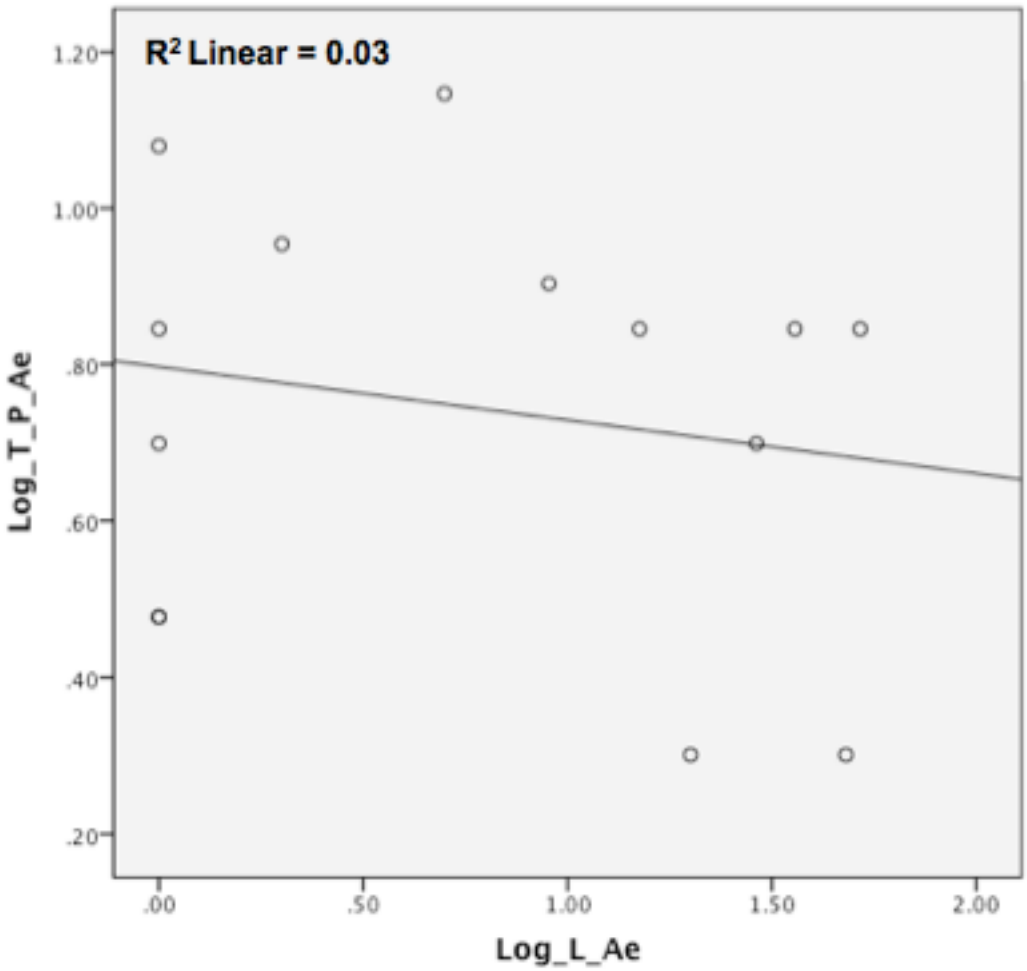


**Figure 6.** Sampled Prokopack densities versus sampled larval densities.

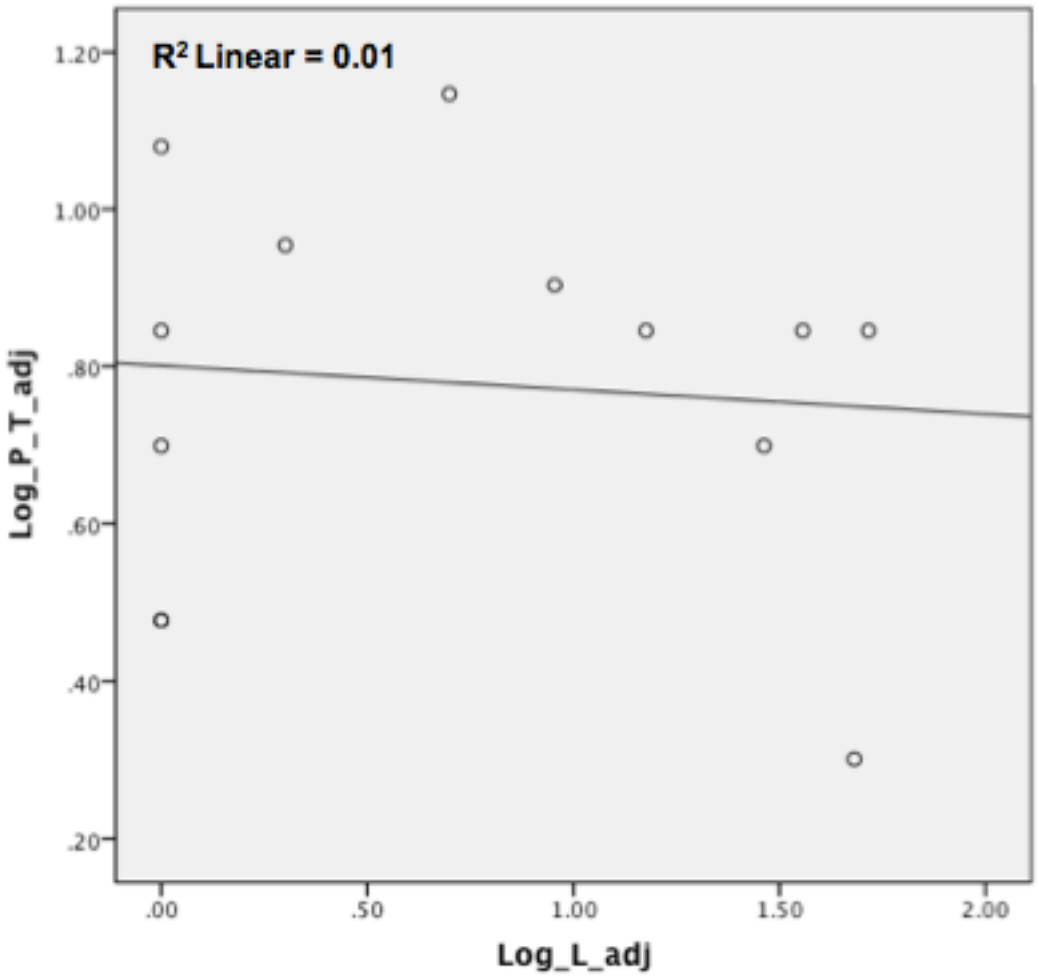


**Figure 7.** Sampled Prokopack densities versus sampled larval densities with censored point.

A similar relationship existed when using outdoor larval densities to predict BGS collection outdoors with a weak linear correlation ($R^2$= 0.05) and not enough evidence ($t_{12}$= 0.77, p= 0.45) that the slope was not zero at the $\alpha$ = 0.05 level of significance (Fig. 8).

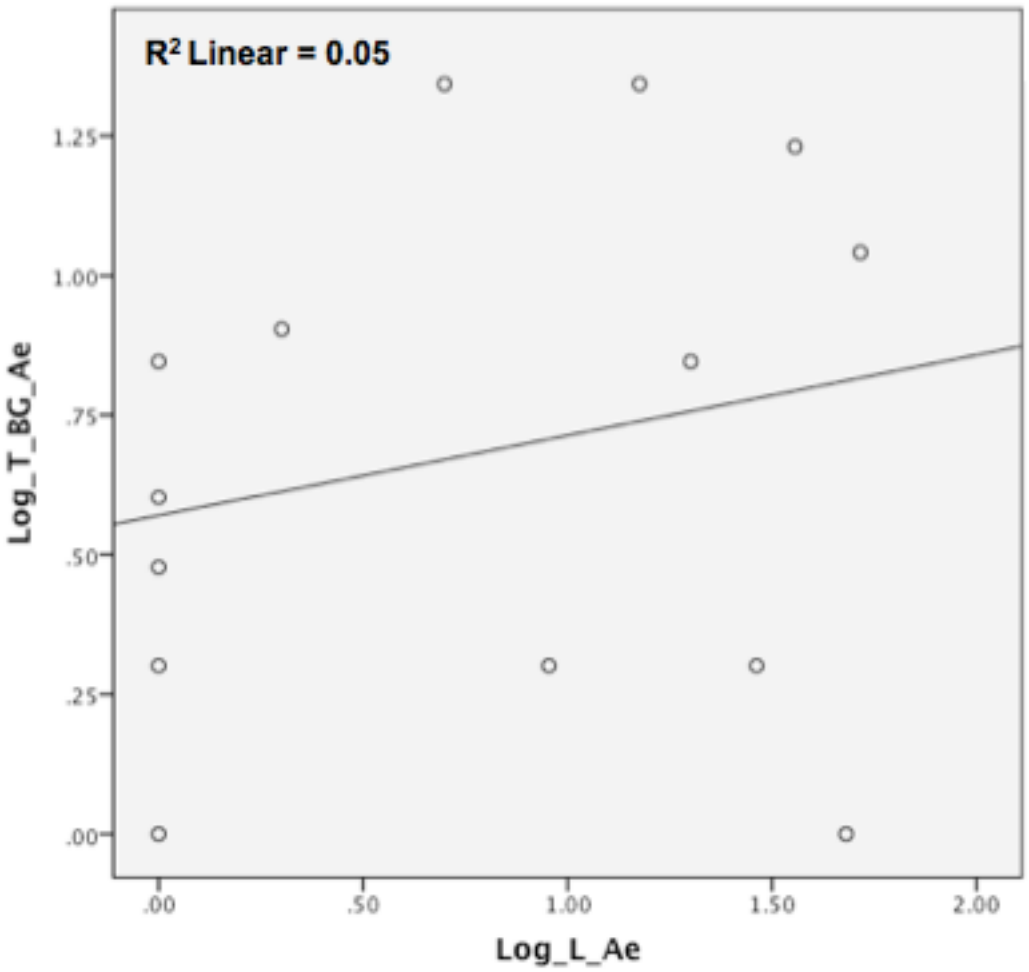


**Figure 8.** BGS sampled densities versus larval sampled densities.

# CHAPTER 5: Domestic Survey

## OUTDOOR LARVAL SURVEY RESULTS

Overall, 83 household plots were surveyed for containers positive with *Ae. aegypti* larvae. The survey yielded 96 *Ae. aegypti* positive containers and 672 *Ae. aegypti* larvae. Of the sampled larvae, 181 (26.9%) were females, 200 (29.8%) were males, and 291 (43.3%) had an undetermined sex (i.e. were not allowed to emerge or died prior to emergence). The number of positive containers, total *Ae. aegypti* larvae, ratio of female to male *Ae. aegypti* larvae, and mean ± SE of *Ae. aegypti* larvae are given in Table 6 for Area 1 and Area 2 are given in Table 6. Five container characteristics were evaluated for larval density: container type, container volume, container construction material, average distance of containers from the house, and shade coverage of the container.

**Table 6.** *Ae. aegypti* larval collection data comparison of Area 1 and Area 2 in Sint Eustatius.

| | Area 1[1] | | | | Area 2[2] | | | |
|---|---|---|---|---|---|---|---|---|
| | No. Positive[1] | *Ae. aegypti*[1] | *Ae. aegypti*[2] ♀ : ♂ | Mean ± Std Error[1] | No. Positive[1] | *Ae. aegypti*[1] | *Ae. aegypti*[2] ♀ : ♂ | Mean ± Std Error[1] |
| **Container Type** | | | | | | | | |
| 1 Garbage Related | 1 | 9 | 6 : 3 | 9.00 ± 0.00 | 44 | 312 | 79 : 82 | 7.09 ± 1.00 |
| 2 Ornamental | 6 | 57 | 19 : 21 | 9.50 ± 3.72 | 12 | 58 | 15 : 19 | 4.83 ± 1.09 |
| 3 Domestic use | 5 | 79 | 20 : 21 | 15.80 ± 7.55 | 27 | 155 | 40 : 54 | 5.74 ± 0.78 |
| 4 Building Foundation | 0 | - | - | - | 0 | - | - | - |
| **Container Volume** | | | | | | | | |
| 1 < 250ml | 5 | 72 | 13 : 9 | 14.40 ± 7.44 | 14 | 78 | 28 : 11 | 5.57 ± 1.39 |
| 2 250ml to 1L | 2 | 32 | 15 : 17 | 16.00 ± 10.00 | 19 | 169 | 40 : 46 | 8.89 ± 1.89 |
| 3 1L to 25L | 4 | 38 | 16 : 17 | 9.50 ± 4.09 | 26 | 181 | 44 : 56 | 6.96 ± 0.77 |
| 4 25L to 1000L | 1 | 3 | 1 : 2 | 3.00 ± 0.00 | 17 | 81 | 18 : 31 | 4.76 ± 0.95 |
| 5 >1000L | 0 | - | - | - | 8 | 18 | 6 : 11 | 2.25 ± 0.59 |
| **Container Material** | | | | | | | | |
| 1 Plastic | 7 | 66 | 22 : 27 | 9.43 ± 2.32 | 62 | 350 | 100 : 116 | 5.65 ± 0.54 |
| 2 Metal | 4 | 53 | 9 : 6 | 13.25 ± 10.00 | 11 | 92 | 13 : 24 | 8.36 ± 3.05 |
| 3 Ceramic or pottery | 1 | 26 | 14 : 12 | 26.00 ± 0.00 | 2 | 10 | 6 : 1 | 5.00 ± 0.00 |
| 4 Rubber | 0 | - | - | - | 8 | 74 | 17 : 14 | 9.25 ± 2.11 |
| 5 Glass | 0 | - | - | - | 0 | - | - | - |
| 6 Fiberglass | 0 | - | - | - | 0 | - | - | - |
| 7 Cement | 0 | - | - | - | 0 | - | - | - |
| 8 Organic | 0 | - | - | - | 0 | - | - | - |
| **Avg. Distance** | | | | | | | | |
| ≤ 1m | 8 | 88 | 35 : 34 | 11.00 ± 3.06 | 37 | 190 | 51 : 58 | 5.14 ± 0.74 |
| 1m ≥ 5m | 4 | 57 | 11 : 11 | 8.36 ± 3.05 | 32 | 241 | 54 : 66 | 7.53 ± 1.25 |
| 5m < | 0 | - | - | - | 15 | 96 | 30 : 31 | 6.40 ± 0.90 |
| **Shade** | | | | | | | | |
| None | 0 | - | - | - | 16 | 74 | 28 : 12 | 4.63 ± 1.26 |
| Partial | 13 | 158 | 48 : 52 | 12.15 ± 3.26 | 25 | 163 | 44 : 51 | 6.52 ± 1.41 |
| Full | 1 | 7 | 2 : 3 | 7.00 ± 0.00 | 33 | 223 | 47 : 74 | 6.76 ± 0.85 |

[1] Ten homes in a neighorhood with homogenous construction and distribution
[2] Seventy-two homes distributed throughout the island with heterogenous construction and distribution
[3] From total larval population regardless of sex
[4] Reflects only larval population where sex could be determined (Area 1= 13.5% and Area 2= 43.2% of total)

Containers were divided into four types: 1) garbage related (discarded tires, buckets, cups), 2) ornamental (flowerpots), 3) domestic use (trashcans, water storage buckets, pet watering dishes), or 4) building foundation (drains). Only three types of containers contained larvae: garbage related (Type 1), ornamental (Type 2), and domestic use (Type 3). Garbage related containers represented 46.9% (45) of the positive containers and resulting in 321 (47.9%) of the larvae in total, where ornamental

containers represented 18.8% (18) positive containers and 115 (17.1%) larvae in total, and domestic use containers represented 33.3% (32) of the positive containers and 234 (34.9%) larvae in total. There was 1 (1.0%) container that held 2 (0.1%) *Ae. aegypti* larvae that was excluded due to no type being recorded.

*Ae. aegypti* larvae were sampled in all size classification of containers: 1) Very Small (<250ml), 2) Small (250ml to 1L), 3) Medium (1L to 25L), 4) Large (25L to 1000L), and 5) Very Large (>1000L). Very small containers represented 19.8% (19) of the positive containers and resulted in 150 (22.3%) *Ae. aegypti* larvae. Small containers (21.9% (21) of positive containers) contained 201 (29.9%) *Ae. aegypti* larvae in. Medium containers (31.2% (30) of positive containers) resulted in 219 (32.6%) *Ae. aegypti* larvae. Large containers represented 18.8% (18) of the positive containers and resulted in 84 (12.5%) of the *Ae. aegypti* larvae. Very large containers only represented 8.3% (8) of the positive containers and 18 (2.7%) of the *Ae. aegypti* larvae.

Of the eight container construction materials: plastic (1), metal (2), ceramic or pottery (3), rubber (4), glass (5), fiberglass (6), cement (7), and organic (8), only the first four types were positive for *Ae. aegypti* larvae. Plastic containers were the most productive with 69 (71.9%) positive containers that contained 416 (61.9%) *Ae. aegypti* larvae. Metal containers contained 145 (21.6%) *Ae. aegypti* larvae in 15 (15.6%) containers. Ceramic and pottery containers contained 36 (5.4%) in 3 (3.1%) containers. While rubber containers contained 74 (11.0%) *Ae. aegypti* larvae in 8 (8.3%) containers. There was 1 (1.0%) container that held 1 (0.1%) *Ae. aegypti* larvae that was excluded due to no construction material being recorded.

Container distance from homes were averaged and classified into three categories: <1 meter, ≥1 meter but ≤5 meters, and >5 meters. Containers <1 meter from the home contained 278 (41.4%) *Ae. aegypti* larvae from 45 (46.9%) containers. Containers ≥1 meter but ≤5 meters contained 298 (44.3%) *Ae. aegypti* larvae from 36 (37.5%) containers. Containers >5 meters contained 96 (14.3%) *Ae. aegypti* larvae from 15 (15.6%) containers.

*Ae. aegypti* larvae were sampled from all three classifications of shade: none, partial, and full. Containers with no shade contained 74 (11.0%) *Ae. aegypti* larvae from 16 (16.7%) containers. Containers with partial shade contained 321 (47.8%) *Ae. aegypti* larvae from 38 (39.6%) containers. Containers with full shade contained 230 (34.2%) *Ae. aegypti* larvae from 34 (35.4%) containers. There were 8 (8.3%) containers excluded that contained 47 (7.0%) *Ae. aegypti* larvae due to shade classification not being recorded.

Further evaluation of container characteristics was accomplished with the use of a standardized subset of the sampled homes. This subset of 10 homes, located in the government housing area was homogenous in regard to house construction, size of peridomestic area, and distance to nearest neighboring home. Houses in this area were classified as Area 1 and were compared to the remaining 72 houses (classified as Area 2) surveyed which were characterized as being heterogeneous in regard to construction, size and distance to neighboring houses.

### INDOOR ADULT SURVEY RESULTS

A total of 81 houses were sampled for adult *Ae. aegypti indoors* using Prokopack aspiration. Of these, 61 (75.3%) houses were positive for adult *Ae. aegypti* and 20 (24.7%) houses were negative for adult *Ae. aegypti*. There were 2 houses not sampled for

adults after withdrawal from the survey following larval sampling. A total of 747 adult *Ae. aegypti* were collected composed of 364 (48.7%) females and 383 (51.3%) males. All sampled adults were pooled by household and analyzed using qPCR for DENV. No pools were found to be dengue positive. The number of *Ae. aegypti* adult positive homes, total adult *Ae. aegypti*, ratio of female to male adult *Ae. aegypti*, and mean ± SE of *Ae. aegypti* adults are given in Table 7 for Area 1 and Area 2.

**Table 7.** *Ae. aegypti* adult collection data comparison of Area 1 and Area 2 in Sint Eustatius.

| | Area 1[1] | | | | Area 2[2] | | | |
|---|---|---|---|---|---|---|---|---|
| | **No. Positive** | ***Ae. aegypti*** | ***Ae. aegypti*** ♀ : ♂ | **Mean ± Std Error** | **No. Positive** | ***Ae. aegypti*** | ***Ae. aegypti*** ♀ : ♂ | **Mean ± Std Error** |
| **Air Conditioning** | | | | | | | | |
| No | 9 | 162 | 95 : 67 | 18.00 ± 7.68 | 43 | 508 | 241 : 269 | 11.81 ± 2.22 |
| Yes | 1 | 5 | 2 : 3 | 5.00 ± 0.00 | 8 | 72 | 26 : 46 | 9.00 ± 4.44 |
| **Screens** | | | | | | | | |
| No | 9 | 163 | 93 : 70 | 18.11 ± 7.65 | 41 | 538 | 249 : 291 | 13.12 ± 2.36 |
| Partial | 1 | 4 | 4 : 0 | 4.00 ± 0.00 | 1 | 20 | 8 : 12 | 20.00 ± 0.00 |
| Yes | 0 | - | - | - | 9 | 22 | 10 :12 | 2.44 ± 0.48 |
| **Pets / Animals** | | | | | | | | |
| No | 3 | 11 | 7 : 4 | 3.67 ± 0.67 | 21 | 182 | 76 : 106 | 8.67 ± 2.25 |
| Yes | 7 | 156 | 90 : 66 | 22.29 ± 9.34 | 30 | 398 | 191 : 209 | 13.27 ± 2.97 |
| **No. Occupants** | | | | | | | | |
| ≤ 2 | 5 | 64 | 40 : 24 | 12.80 ± 9.06 | 21 | 251 | 91 : 160 | 11.95 ± 3.14 |
| 2 ≥ 4 | 5 | 103 | 57 : 46 | 20.60 ± 11.40 | 21 | 191 | 105 : 86 | 8.90 ± 3.24 |
| 4 < | 0 | - | - | - | 8 | 138 | 69 : 69 | 17.25 ± 4.33 |
| **Doors & Windows** | | | | | | | | |
| Never Open | 0 | - | - | - | 3 | 4 | 4 : 0 | 1.33 ± 0.33 |
| Sometimes Open | 2 | 6 | 6 : 0 | 3.00 ± 1.00 | 7 | 54 | 28 : 26 | 7.71 ± 2.85 |
| Always Open | 8 | 161 | 91 : 70 | 20.13 ± 8.37 | 41 | 522 | 235 : 289 | 12.73 ± 2.38 |
| **Roof Construction** | | | | | | | | |
| Metal | 8 | 158 | 91 : 67 | 19.75 ± 8.47 | 31 | 476 | 213 : 263 | 15.35 ± 2.98 |
| Concrete | 2 | 9 | 6 : 3 | 4.50 ± 0.50 | 19 | 85 | 43 : 42 | 4.47 ± 1.06 |
| Multiple | 0 | - | - | - | 1 | 19 | 10 : 9 | 19.00 ± 0.00 |

[1] Ten homes in a neighorhood with homogenous construction and distribution

[2] Seventy-two homes distributed throughout the island with heterogenous construction and distribution

Six housing characteristics were evaluated as potential risk factors for adult *Ae. aegypti* harborage: presence of air conditioning, window and/or door screens, presence of pets or animals, the number of occupants, whether doors and windows were open, and type of roof construction. No air conditioning was present in 44 (72.1%) homes while 17 (27.9%) had air conditioning. Screens were not present in 50 (82.0%) of the homes, 2 (3.3%) had partial screens, and 9 (14.7%) had fully screened doors and windows. Pets and animals were not present in 24 (39.3%) of the homes while 37 (60.7%) had pets or animals present. The number of occupants was 2 or fewer in 26 (42.6%) of the homes, between 3 or 4 in 26 (42.6%) of the homes, 8 (13.1%) homes had more than 4 occupants, and 1 (1.7%) home did not have the number of occupants recorded. Doors and windows were never left open on 3 (4.9%) homes, sometimes open on 9 (14.8%) homes, and always open on 49 (80.3%) homes. Roof construction consisted of 39 (63.9%) homes with metals roofs, 21 (34.4%) with concrete roofs, and 1 (1.7%) that had a mix of metal and concrete materials. Home characteristics were again further evaluated with the use of a standardized subset of the sampled homes. The same areas indicated in the previous description were used for Area 1 and Area 2.

To analyze risk factors for adult and larval *Ae. aegypti* in an Area1 and Area 2 comparison, six characteristics were selected (Table 8). The data was Log10 (x+1) transformed to meet normality requirements and then independent samples t-tests were performed on container density, average container distance, larval density, human density, average distance to nearest house, and adult density. Average container distance ($t_{39}$= -2.07, p= 0.05) and human density ($t_{17}$= -2.27, p= 0.04) were significantly different at the $\alpha$= 0.05 level of significance indicating that these two factors contribute to the

differences between these areas. Container density ($t_{18}$= -0.47, p= 0.66), larval density ($t_{12}$= 0.74, p= 0.48), average distance to nearest house ($t_{25}$= -0.51, p= 0.62), and adult density ($t_{13}$= 1.30, p= 0.22) did not differ significantly at the $\alpha$= 0.05 level of significance indicating that these four factors do not contribute to the differences between these areas.

**Table 8.** Comparison of Area 1 and Area 2 factors of adult and larval *Ae. aegypti*

| | Mean | sd | t-test[1] | df | p-value |
|---|---|---|---|---|---|
| **Container Density** | | | | | |
| Area 1[1] | 1.09 | 1.04 | -0.47 | 18.00 | 0.64 |
| Area 2[2] | 1.26 | 1.61 | | | |
| **Avg. Container Distance** | | | | | |
| Area 1[1] | 0.63 | 1.04 | -2.07 | 39.00 | 0.05 |
| Area 2[2] | 1.58 | 2.83 | | | |
| **Larval Density** | | | | | |
| Area 1[1] | 0.69 | 0.69 | 0.74 | 12.00 | 0.48 |
| Area 2[2] | 0.53 | 0.59 | | | |
| **Human Density** | | | | | |
| Area 1[1] | 2.18 | 1.08 | -2.27 | 17.00 | 0.04 |
| Area 2[2] | 3.03 | 1.55 | | | |
| **Avg. Distance to Nearest House** | | | | | |
| Area 1[1] | 11.82 | 6.03 | -0.51 | 25.00 | 0.62 |
| Area 2[2] | 13.00 | 10.83 | | | |
| **Adult Density** | | | | | |
| Area 1[1] | 0.85 | 0.58 | 1.30 | 13.00 | 0.22 |
| Area 2[2] | 0.61 | 0.55 | | | |

[1] Log10 (x+1) transformed indepent samples t-test with equal variances not assumed.

[2] Ten homes in a neighorhood with homogenous construction and distribution

[3] Seventy-two homes distributed throughout the island with heterogenous construction and distribution

## CORRELATIONS

Multiple linear regressions were performed for the total adult and larval *Ae. aegypti* in the entire survey area. Before analysis, the data were $Log_{10}$ (x+1) transformed to meet normality requirements. The larval model included container volume, type, material, and average distance from house and produced a weak linear relationship ($R^2$= 0.11). At the $\alpha$ = 0.05 level of significance there was not enough evidence ($F_4$= 3.17, p=

0.02) that the slope was not zero and these factors combined contributed significantly to the larval populations.

The adult model included presence of air conditioning, whether windows and/or doors were screened, presence of pets or animals, the number of occupants, whether doors and windows were open and frequency, and distance to nearest house. The multiple linear regressions produced a positive linear relationship ($R^2 = .42$). At the $\alpha = 0.05$ level of significance there was statistically significant evidence ($F_4 = 7.01$, $p = 0.00$) that these factors contributed to the positive linear relationship of adult *Ae. aegypti*.

**GIS ANALYSIS**

Statistical analysis of SRTM DEM data in ArcGIS showed a mean elevation of homes positive for *Ae. aegypti* was 60.54 meters ± 23.10 standard deviation (SD) with a range of 26 – 126 meters. The same analysis of homes negative for *Ae. aegypti* had 74.97 meters ± 26.13 SD with a range of 39 – 129 meters. Given these results it is likely that elevation is not a significant factor in the distribution of *Ae. aegypti* in Sint Eustatius.

Georeferenced collection data allowed for the creation of two maps in ArcGIS. These two maps depict collection site values overlaid IDW predicted vales for sites not sampled. The collection sites are represented with symbols representing the categorical values sampled at the site. Scaled shading represents the predicted vales, with low density represented by the light regions and higher density by the darker shades. The adult map is a representation of the collected adult females versus the predicted adult population (Fig. 9). The larval map represents the number of larval positive containers at each site versus the predicted number of positive larval containers (Fig. 10).

For this study the analysis of dengue with sampled *Ae. aegypti* was not possible with GIS. Georeferenced data was not available for prior confirmed cases of dengue to overlay with collection data. Results from the PCR analysis of sampled adult *Ae. aegypti* also did not identify any dengue positive pools for analysis.

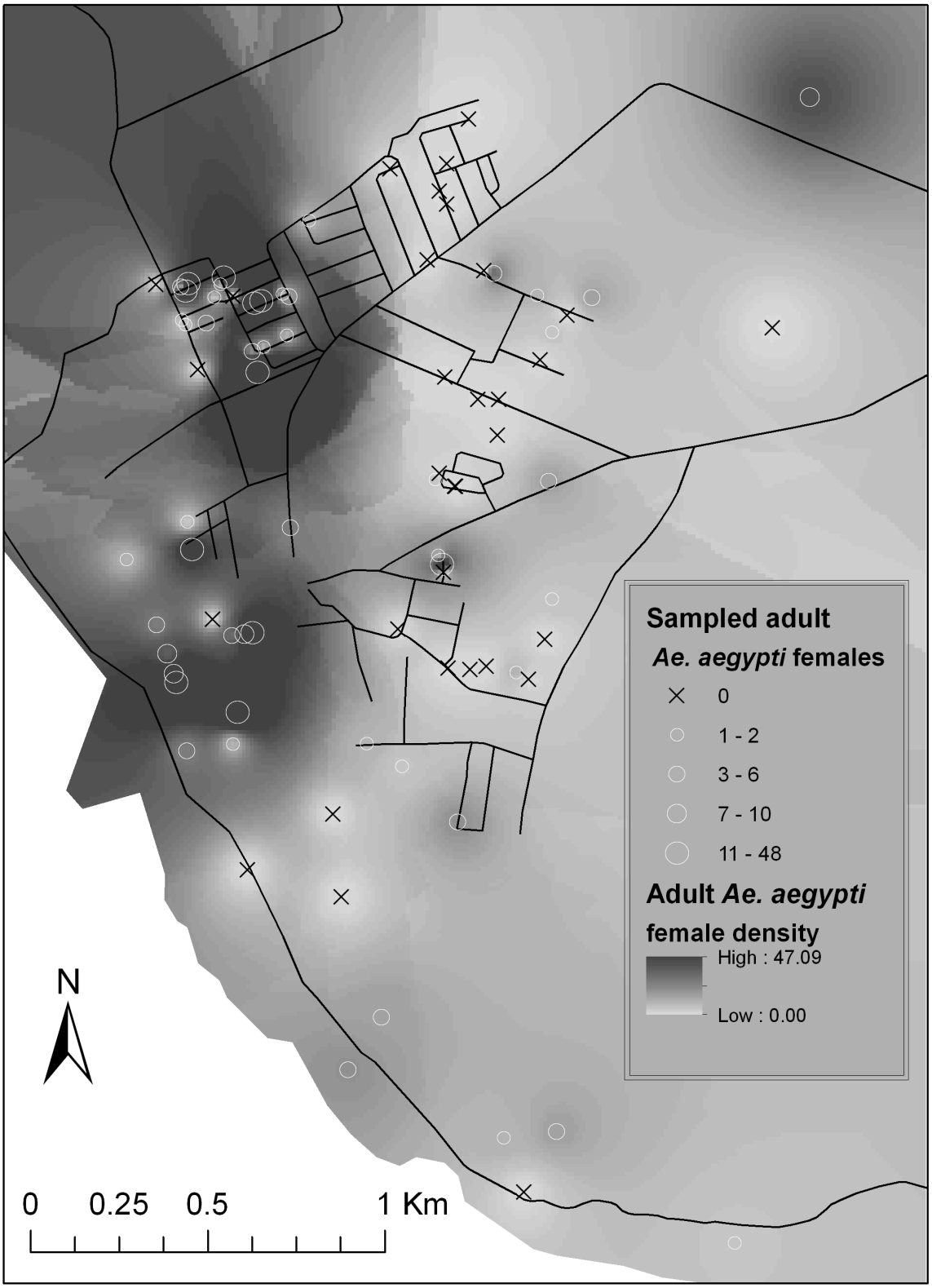


**Figure 9.** Sampled adult female *Ae. aegypti* layered with predicted female *Ae. aegypti*.


**Figure 10.** Sampled *Ae. aegypti* positive containers layered with predicted positive containers layered *Ae. aegypti*.

# CHAPTER 6: Discussion

The goal of this project was to characterize the distribution of *Aedes aegypti* on the island of Sint Eustatius using both Prokopack aspiration and the BioGents-Sentinel™ mosquito trap and identify potential risk factors for the distribution of adult *Ae. aegypti* and dengue infections. Results provide evidence for the effectiveness of both the Prokopack and BGS trap for sampling *Ae. aegypti,* inside and outdoors of homes, respectively.

## SAMPLING TECHNIQUE COMPARISON

Although the BGS captured a greater number of *Ae. aegypti* as compared to Prokopack aspiration, this difference was not statistically significant. Both methods were equally efficient at capturing both male and female *Ae. aegypti*. Support of these findings is the regression model that indicates a positive linear relationship between the two sampling methods. These results agree with the findings of Williams et al. (50) in northern Queensland, Australia that the BGS was effective for *Ae. aegypti* sampling and those of Vazquez-Prokopec et al. (47) that validated the Prokopack was as effective as the "gold standard" CDC Backpack Aspirator. Given the efficiency and similar results of the two methods, integration of either method into an adult surveillance program in St. Eustatius could be beneficial on this island.

Regression analysis indicated that adult *Ae. aegypti* sampling densities from outdoor BGS trapping can estimate indoor adult densities. While this positive relationship existed between BGS and Prokopack (i.e., adult mosquito populations), there was not a linear relationship for BGS and outdoor larval densities. The same was true for

Prokopack and outdoor densities of *Ae. aegypti* larvae. These results indicate that, in this study, larval sampling was not predictive of adult densities. If the purpose of the current larval surveillance in Sint Eustatius was the prediction of adult densities, then the linear relationships demonstrate that BGS trapping would be more effective for estimating adult populations. These findings are supported by previous reports that larval sampling is not a good predictor of adult density due to biological factors that affect *Ae. aegypti* productivity of different containers (56).

Though this study's findings suggest that the BGS is as effective at estimating indoor *Ae. aegypti* adult densities as the already proven Prokopack, the objective of sampling must be considered. If the purpose of surveying is to estimate *Ae. aegypti* population indoors or outdoors, then outdoor BGS trapping is a capable surrogate for indoor sampling. If the purpose of the survey is to collect dengue virus-infected *Ae. aegypti*, the Prokopack may be more appropriate as it targets the collection of mosquitoes that are resting indoors and are more likely to have taken a human blood meal. Likewise, the availability of resources must also be considered. These resources include labor, equipment, equipment maintenance, training, and their associated costs. While not directly measured in the current study, an optimum surveillance method in a developing country would include effectiveness while being inexpensive and requiring minimal man-power.

The Prokopack in this study was capable of capturing over 50 *Ae. aegypti* per man hour, representing a sampling time of 1.5 hours per house, while the BGS trap only captured 1.7 *Ae. aegypti* per trap hour, representing a total of 56.4 hours for all houses sampled. Despite the overall discrepancy in total *Ae. aegypti* density collected between

the two sampling methods, these results only take into consideration the amount of time the devices were actually functioning and do not consider the additional labor requirements that were required for conducting the survey. The Prokopack requires a trained operator to enter homes and actively sample each room for a designated time period. The BGS, on the other hand, only requires a trained operator to set the trap at the prescribed location(s) and return to collect the trap at the end of the sampling period. The difference then is Prokopack sampling requires constant manpower to complete the survey while BGS allows for a period during the survey that the operator can perform other tasks. When there are manpower and funding limitations, the ability to have operators available to perform more than one task provides a cost savings and may support the use of this method.

Availability of equipment may represent the biggest hurdle to the use of either sampling method. Between the two methods evaluated in this study, the Prokopack has a cost of about $75 USD while the BGS has a cost of about $250 USD. Both devices can employ the same power supply and therefore would have the same initial and maintenance costs with regard to battery supply. At over 3 times the cost of the Prokopack, the initial cost alone may deter the employment of the BGS for *Ae. aegypti* sampling in developing countries. In order to be effective, the BGS also requires an attractant lure, which is suggested to be replaced every 3 to 5 months, at a cost of approximately $30 USD. However, over time, the BGS could offer a savings on labor that may offset these initial and maintenance supply costs. The durability of both the Prokopack and BGS was not evaluated. It would be necessary in a resource-limited setting to evaluate the cost of maintenance and replacement of these devices. Both

methods have similar training requirements for their effective use; however, there exists a difference in the efficiency between the methods due to the nature of the sampling. As noted by Williams et al. (50), operator performance with the Prokopack influences mosquito collection success as each collector will have an inherent bias as to how they sample with the device. For this reason, the BGS may offer more standardized results. Based on the results of the current study and taking overall cost and data standardization into consideration, the use of the BGS for estimation of indoor adult *Ae. aegypti* populations is indicated as a viable option. BGS trapping offers a significant benefit over the use of larval surveys, if adult *Ae. aegypti* population surveillance is the purpose of activity.

**DOMESTIC SURVEY**

This study characterized the types of containers positive for immature stages of *Ae. aegypti* in St. Eustatius. The findings show that garbage and domestic use containers produced 95% of the total *Ae. aegypti* larvae collected. These findings agree with Gubler (12) that artificial containers for domestic use and garbage are preferred *Ae. aegypti* habitats. Plastic containers held almost three times the number of larvae of all other container construction materials combined and the majority of positive containers occurred within a distance of 5 meters of the home. What these results indicate is that humans were responsible for the greatest proportion of aquatic habitats positive for *Ae. aegypti* mosquitoes during the current survey. Given this, a clean-up program could help eliminate habitats. If the data is representative of the year-round environment, application of this strategy in a 5-meter radius around homes could eliminate almost three-quarters of larval habitat.

At the time of this survey, there was active larviciding by the Sint Eustatius Department of Public Health (DPH). The Sint Eustatius DPH control and monitoring strategy for mosquitoes consists of identifying larval positive containers, treating mosquito positive containers with Bti or temephos, and then recording the treatment data. Even with this active program, over half of the homes surveyed were positive for immature *Ae. aegypti*. The limitation of this program is that there is only one public health official that is involved with the larviciding campaign and this individual is also responsible for other duties.

Cisterns and gutters were omitted from sampling, as they are part of the water supply for the majority of the homes in Sint Eustatius. Given the findings of Medronho et al. (21), where these sites contributed significantly to the breeding habitat *Ae. aegypti*, an assessment of these water sources would be highly recommended. Results presented from the current study cannot be used to determine the container characteristics contributing to the larval population. It should also be noted that this survey did not characterize containers that were negative. Not including the negative containers and their characteristics omitted data necessary to correctly construct a predictive model. In addition, the current survey did not address the productivity of the sampled containers. Future studies should include an assessment of the container density and container productivity for a complete understanding of optimal *Ae. aegypti* habitats in Sint Eustatius.

Three out of every four homes surveyed on Sint Eustatius were positive for adult *Ae. aegypti*. Homes with metal roof construction had mean adult *Ae. aegypti* nearly four times higher than those of homes with concrete construction. This most likely is due to

an association with indoor temperature and/or humidity. Homes with metal roofs are generally cooler than those constructed entirely of cement. This increased indoor temperature in cement homes may make the structure less suitable for resting locations. Although *Ae. aegypti* exhibit anthropophilic behaviors, higher mean values were associated with homes with pets and animals present and may suggest some other association besides feeding behavior.

While the presence of air conditioning did not eliminate *Ae. aegypti* indoors, in positive homes with air conditioning there was a lower mean number of adult mosquitoes collected. The air conditioners used at this location were single room units that were usually only installed in the bedroom(s) of homes and most residents only utilized their air conditioners at night when they were sleeping. For this reason, the windows and doors were open for a portion or entire day in a portion of the homes with air conditioning. A better measure for the relationship between use of air conditioning and presence or abundance of *Ae. aegypti* adults would have been "if" or "when" air conditioning was used. Further analysis is required to understand the effect of air conditioning in this manner.

The presence of window and door screens, as well as whether the portals were closed, resulted in similar findings to that seen in homes with air conditioning whereby this did not correlate to an absence of adult *Ae. aegypti* but did result in lower mean adult populations collected. The majority of screens installed on windows were not of commercial production but rather represented improvised construction (i.e. tacked or nailed to a wooden frame) and were often damaged. The issue with the installation of these wooden framed screens was that they often had considerable space around the

window that allowed for mosquito entry. The other issue was that only one home had a screen door and it was of a sliding construction that did not completely seal. The presence of screens did not completely prevent the entry of mosquitoes into the home.

Unlike prediction of larval *Ae. aegypti* populations, house factors characterized in the current study were able to be used to indicate a positive relationship with presence of mosquitoes. These factors included presence of pets and animals, number of occupants, distance to nearest house, presence or absence of screens, use of air conditioning, and windows and doors being open. However, given the reduction of *Ae. aegypti* adults collected in homes with air conditioning and screens, the model needs further validation prior to use for population estimates. However descriptive statistics have provided a reasonable starting point for prediction of adult *Ae. aegypti* populations and at a minimum, these house characteristics can be used to prioritize which homes are likely harborages of mosquitoes and therefore require monitoring.

Only two of the characteristics used to determine risk of *Ae. aeygpti* presence between Area 1 and Area 2 were predictive. Of those, the significant difference of average distance of containers from the home was likely a function of the difference in the size the plots between the areas. In Area 1, all the homes had similar plot sizes where in Area 2 the plot sizes were variable and the actual size was often undefined. In addition, the significant difference in human density was likely a function of the variability of the size of the homes in Area 2. Homes in Area 1 had only 1 bathroom and 3 bedrooms, which may have served to limit the number of residents in comparison to Area 2. Overall, there was no significant difference in risk between the two areas surveyed. However, this may indicate the need to include more characteristics related to

presence of *Ae. aegypti* and highlights the importance of a thorough understanding of the ecology of mosquitoes at the site of interest.

The current study demonstrated that spatial analyses can be used to model risk for *Ae. aegypti* adult and larval densities. However, the predicted densities of adults and larvae did not overlay. The assumption could be made that the maps are inaccurate however consideration should first be given to what is being mapped, what is known about the area, and how other factors may influence the maps. Several factors could be responsible for this disparity. The first consideration is that the study represented a single time period. Longitudinal sampling would be required to provide a more robust dataset and thereby development of risk models. Another limitation is that only those containers positive for *Ae. aegypti* larvae were recorded. Both positive and negative containers will need to be identified for refined risk mapping. Cryptic breeding sites, such as gutters and cisterns, were also not sampled and inclusion of this data could greatly alter the model as well as environmental influences of temperature, humidity and wind. Wind contribution though is likely minimal as the prevailing winds during the study were perpendicular to that of the representation (52). In addition, container productivity was not characterized therefore the model produced here may not be accurate for predicting larval densities. While further examination of each of these assumptions is required for validation of the risk map produced here, it is likely that, for this survey, the map provides an accurate depiction of the presence of positive larval habitats of *Ae. aegypti* in Sint Eustatius and that GIS technologies can be used as part of a dengue risk assessment system.

Lastly, during the time period of this survey, no dengue virus positive mosquito pools were found. If the sample is representative of the distribution of *Ae. aegypti* on the

island, then it is likely that dengue is not endemic in Sint Eustatius. However, this study was conducted during the beginning of the wet season, prior to when peak mosquito populations would be expected to be present. In addition, due to the lack of previous transmission information from the island, it is difficult to know when the peak transmission season for dengue virus may occur. The lack of georeferenced human or mosquito dengue infection data limits the ability of this study to evaluate *Ae. aegypti* risk factors with regard to virus transmission. For this reason, it is suggested that future studies be conducted throughout multiple seasons to provide longitudinal surveillance data.

# Chapter 7: Conclusion

This study represents the first known entomological research survey in Sint Eustatius in over 65 years. The last known published survey was in 1947 when Edwin van der Kuyp surveyed the island while surveying mosquitoes of the Netherlands Antilles (18). It is likely that this is the first study that focused on the characterization of the spatial distribution of larval and adult *Ae. aegypti* in Sint Eustatius for dengue transmission. In addition, this study also likely represents the first evaluation of the efficiency of methods for adult collection at this location.

Anecdotally, no *Ae. albopictus* were collected in any of the homes or traps during this survey. While this may imply that the species is absent from the island, further investigation is required for determination if the species is truly absent. It is likely that since this survey focused primarily on urban and suburban residences, excluding business locations, and sampled limited rural locations, the species may be present but has yet to displace *Ae. aegypti* in residential settings.

Validation for the use of Prokopack and BioGents-Sentinel™ mosquito trap for sampling both indoor and outdoor *Ae. aegypti*, respectively, in Sint Eustatius was provided in this study. The findings provide strong evidence for the use of the BGS trap for the estimation of indoor *Ae. aegypti* populations at this location. Regardless, the purpose for sampling must be considered prior to selection of method as these devices sample two distinct populations of the adult *Ae. aegypti*.

The study demonstrated that the most prevalent *Ae. aegypti* oviposition sites surveyed were domestic use and garbage-related containers whose volume was less than

25 Liters. The most common location of these sites was within 5 meters of homes and largely consisted of plastic material. When these containers were partially or fully shaded they were significantly more productive. A clean-up program is highly suggested to target these habitats. For the containers that cannot be disposed of, proper storage to prevent water collection or covering to prevent mosquito entry is recommended.

Risk factors for indoor adult *Ae. aegypti* were found to be the absence of air conditioning, lack of window and door screens, presence of pets or animals, the frequency doors and windows being open, and roof construction material. Conversely, there was no single factor found to coincide with the complete absence of adult *Ae. aegypti* indoors in Sint Eustatius at the time of this survey. However, window and door screens, keeping windows and doors closed, and use of air conditioning were the conditions under which the lowest mean *Ae. aegypti* indoor values were indicated.

Perhaps most significant, careful consideration must be given to the limited resources of Sint Eustatius before implementing an *Ae. aegypti* surveillance program. Being a small Caribbean island, there are supply limitations for equipment and parts and limited manpower for surveillance and control. Given that there was an active larviciding program at the time of this survey and the sampled densities, these very limitations may be having a negative impact on the current control strategy. These limited resources also impact the potential effectiveness of the use of GIS to predict at-risk locations in Sint Eustatius, as it is cost-prohibitive and requires technical expertise. If resources were made available, the reduction in larval habitats resulting from a clean up campaign could be

monitored using GIS to further optimize resource allocation by targeting control efforts in high-risk areas.

While both the BGS and Prokopack are efficient sampling tools, their use may be limited due to cost. However, based on results presented here, the BGS is indicative of providing the ability to sample outdoor populations and estimate indoor populations thereby saving on labor and not requiring entrance into residents' homes, one major challenge to indoor adult surveillance. Perhaps most important, the potential impact of early dengue outbreak detection using a validated sampling method, such as the BGS, to prevent a major epidemic may override the associated costs of surveillance. For these reasons, the implementation of *Ae. aegypti* surveillance programs and control strategies require further evaluation to identify optimum use of resources.

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# APPENDICES

## APPENDIX 1

### House Characterization

Sample Number: _____ Household Number: _____ Date: _____/_____/_____

Neighborhood: _____ Coordinates: ____°____.____" N ____°____.____" W

Mapping Team: _____ _____

---

# of Buildings: _____ Elevated House: Y / N Air Conditioning: Y / N

Windows: #_____ # Open _____ # Closed _____ Screens: Y / N

Doors: #_____ # Open _____ # Closed _____

Construction: Concrete or Masonry / Wood / Other: _____

Floor: Planks / Tile / Concrete / Dirt / Other: _____

Roof: Concrete / Metal / Asphalt / Shingle / Tile / Other:_____

Types & # of Animals Present: _____ _____

Water Source: Municipal / Closed Storage Tank / Open Storage Tank / Undetermined

Refuse Water: Municipal / Closed Septic Tank / Open Septic / Undetermined

Distance to Nearest House(s) (Meters):_____ _____

Notes:

**APPENDIX 2**

## Prokopak Mosquito Collection

Sample Number: _____ Household Number: _____ Date: _____/_____/_____

Neighborhood: _____ Coordinates : ____°____.____' N ____°____.____' W

Start Time (24H): _____:_____ End Time (24H): _____:_____ Duration: _____:_____

Collectors:

_____

Insecticide Usage: Y / N Insecticide/Product Name:_____

Last Application Date:_____/____/_____ Applied by: Homeowner / Professional

Frequency of Application: Daily / Weekly / Monthly / Other:_____

# of Occupants (Residents):_____ # of Rooms:_____ # of Rooms Sampled:_____

Windows: Always Open / Only During Day / Only During Night / Never Open

Doors: Always Open / Only During Day / Only During Night / Never Open

Cloud Cover: Clear / Fog / Partly Cloudy / Cloudy / Full Overcast

| Numbers Collected | Females | Males |
|---|---|---|
| Ae. aegypti | | |
| Ae. albopictus | | |
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NOTES:

## APPENDIX 3

Statia Larval Mosquito Habitat Survey

Sample Number: _____ Household Number: _____ Neighborhood: _____ Date: ___/___/_____

Coordinates : ___ ° ___.___ 'N ___ ° ___.___ 'W Start Time (24H): ___:___ End Time (24H): ___:___ Duration: ___:___

Collectors: _____

Precipitation: None / Light / Drizzle / Rain / Heavy Surrounding Terrain: Mountain / Valley

Vegetation in House Vicinity: Ornamental / Trees / Grass / Agriculture / Undetermined

---

**Container Type:** 1 Garbage Related (Discarded tires, buckets, cups), 2 Ornamental (Flower Pots), 3 Domestic Use (Trash Can Lids), or 4 Building Foundation

**Container Volume:** 1 Very Small (<250ml), 2 Small (250ml to 1L), 3 Medium (1L to 25L), 4 Large (25L to 1000L), or 5 Very Large (>1000L)

- If volume cannot be determined the dimensions in centimeters can be recorded for volume calculation

**Container Construction:** 1 Plastic, 2 Metal, 3 Ceramic or Pottery, 4 Rubber, 5 Glass, 6 Fiberglass, 7 Cement, or 8 Organic

**Container Height:** Container opening distance above the ground

**Distance from House:** Measurement from closest entry point in meters

**Shade:** 1 No Shade, 2 Partial, 3 Full

**Aquatic Vegetation Present:** 1 Submerged, 2 Floating, 3 Emergent, 4 Sub & Float, 5 Sub & Emerg, 6 Float & Emerg, 7 All types

| Collection ID # | Container Type | Container Volume | Container Construction | Container Height | Distance From House | Shade | Aquatic Vegetation Present | # Larvae Present | # Ae. aegypti Male | # Ae. albopictus Females | # Ae. albopictus Males | Other Female | Other Male |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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**APPENDIX 4**

## BGS Mosquito Collection

Sample Number: _____ Household Number: _____ Date: _____/_____/_____

Neighborhood: _____ Coordinates: _____°_____._____' N _____°_____._____' W

Start Time (24H): _____:_____ End Time (24H): _____:_____ Duration: _____:_____

Collectors:

_____

_____

Precipitation: None / Light / Rain / Heavy

Cloud Cover: Clear / Fog / Partly Cloudy / Cloudy / Full Overcast

---

BGS Trap ID#_____ Battery ID#_____

| Numbers Collected | Females | Males |
|---|---|---|
| Ae. aegypti | | |
| Ae. albopictus | | |
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NOTES: